SYMPOSIUM ON NUCLEAR SEX

Edited for the Organizing Committee by the Secretary

D ROBERTSON SMITH M.A M D

and

WILLIAM M DAVIDSON M D

Department of Chemical Pathology,
King's College Hospital Medical School,
London

Foreword by

Professor Robert Platt, M D

President of the Royal College of Physicians



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PREFACE

TOWARDS the end of 1956 it was learned that Professor Murray Barr intended to come to this country during the following Summer To give people interested in nuclear sex an opportunity to meet him, Dr Bernard Lennox suggested organising a meeting A preliminary enquiry revealed considerable interest in such a project and a committee was formed to make the necessary arrangements.

It seemed most profitable to embrace in a Symposium all the fields with interests related to nuclear sexing including Anatomy Biochemistry Biology Clinical Medicine Cytology Endocrinology Genetics, Pathology Physiology Psychology and Surgery and a number of representatives were invited with this in view

The Symposium was held in September 1957 at King's College Hospital Medical School at the invitation of the Dean and Council The scientific proceedings, to which the participants were welcomed by Dr William M. Davidson consisted of three sessions of papers and discussions and a number of demonstrations illustrating related points. The proceedings were summarised and concluded by Professor Murray Barr

As much of the material presented at the Symposium was new and likely to be of wide interest it was decided to collect the papers and the recorded discussions into book form.

We wish to acknowledge here our gratitude to those who made this Symposium possible the Dean and Council of King's College Hospital Medical School, the Board of Governors of King's College Hospital, the Wellcome Trust and the Ciba Foundation

We are also greatly indebted to Mr W F Gunn the Secretary of the Medical School and his Staff for their assistance in organising the meeting Dr F L. Jackson for recording the proceedings, and the secretarial and technical staffs of the Pathological Department who helped in so many ways to make the Symposium a success.

Finally we would like to record our appreciation of the valuable help given to us in the preparation of these proceedings for publication by Mr Owen R. Evans of Heinemann Medical Books Limited

D ROBERTSON SMITH
WILLIAM M. DAVIDSON

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The Symposium was held at King's College Hospital Medical School, London, S.E.5, on September 6th and 7th, 1957, by kind permission of the Dean and Council of the Medical School.

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DELEGATES ATTENDING SYMPOSIUM ON NUCLEAR SEX

ARMSTRONG, Dr C. N.
Royal Victoria Infirmary
Newcastle upon Tyne 1

ASHLEY Dr D J B
David Lewis Northern Hospital
Liverpool 3

ATKIN Dr N B.
Dept of Cancer Research,
Mount Vernon Hospital
Northwood, Middlesex.

BARR, Prof. Murray L.
The University of Western Ontario
Faculty of Medicine
346 *South Street*
London, Canada

BENSTED Dr J P M
Dept. of Pathology
Guy's Hospital
London, S.E 1

BISHOP Dr P M. F
22 *Harley Street,*
London, W 1

BROTTER, Mr L. R.
75 *Harley Street*
London W

CARDELL, Dr B. S.
Dept. of Morbid Anatomy
King's College Hospital Medical School
London, S.E.5.

DAVIDSON Dr W M.
Dept. of Clinical Pathology
King's College Hospital Medical School
London, S.E 5.

EMERY Dr J L.
The Children's Hospital
Sheffield, 10

FERGUSON-SMITH Dr M. A.
Dept. of Pathology
Western Infirmary
Glasgow

FERREIRA, Dr H
Dept. of Pathology
Chelsea Hospital for Women
London S W 3

FORD D C. E.
Radiobiological Research Unit,
Atomic Energy Research Establishment
Harwell Berkshire

GRAY Prof. C.
Dept. of Chemical Pathology
King's College Hospital Medical School
London, S.E.5.

GRAY Dr J E.
Dept. of Anatomy
Medical School,
Newcastle upon Tyne 1

GREENE, Dr Raymond
106 *Harley Street*
London W 1

GRESHAM, Dr G. A.
Dept. of Pathology
Cambridge

HALL, Dr V.
King's College Hospital Medical School
London, S.E.5

HAMERTON, Mr J. L.
Natural History Museum
South Kensington,
London, S.W.7

HARRIS, Dr H.
London Hospital Medical School
London, E.1

HUGGETT, Prof. A. St. G.
Dept. of Physiology
St. Mary's Hospital Medical School,
London, W.2

IZATT, Miss Marian
Blood Transfusion Service
Law Hospital
Carluke, Lanarkshire

JOST, Prof. A.
Laboratoire de Physiologie Comparée
12, Rue Cuvier
Paris 5, France

KLINGER, Mr H. P.
Anatomisches Institut der Universität Basel,
Pestalozzistrasse 20
Basle, Switzerland

LAWLER, Dr Sylvia
Galton Laboratory
University College
London, W.C.1

LENNOX, Dr Bernard
Dept. of Pathology
Western Infirmary
Glasgow, W.1

LEROY, Dr M. H.
50 Fountayne Road,
London, N.16.

LEUCHTENBERGER, Dr Cecilie
Western Reserve University
Cleveland,
Ohio, U.S.A.

LEUCHTENBERGER, Dr R.
Western Reserve University
Cleveland
Ohio, U.S.A.

LIVINGSTONE, Dr J. L.
King's College Hospital
London, S.E.5.

MARBERGER, Dr Eve
Haspingerstrasse 12
Innsbruck, Austria

MITTWOCH, Dr Ursula
Galton Laboratory
University College
London, W.C.1

MYERS, Dr Lois
Dept. of Pathology
Middlesex Hospital
London, W.1

OVERZIER, Prof. C.
Universität Klinik,
Mainz, Germany

PENROSE, Prof. L. S.
The Galton Laboratory
University College
London, W.C.1

PLATT, Prof. Robert
Dept. of Medicine
The University
Manchester 13.

POLANI, Dr P E.
Keats' House
Guy's Hospital
London, S.E.1

PRADER, Dr A.
Kinderspital,
Steinwiesstrasse
Zurich Switzerland.

RACE, Dr R. R.
Blood Group Research Unit,
The Lister Institute
Chelsea Bridge Road,
London, S W 1

REES, Mr Harland
King's College Hospital
London, S.E 5.

RIIS, Dr P
Jaegersborgalle 211
Gentofte Denmark

SACHS, Dr L.
The Weizmann Institute of Science
Rehovoth, Israel

SANGER, Dr Ruth
Blood Group Research Unit
Lister Institute
Chelsea Bridge Road,
London, S W

SCHWARZACHER, Dr H. B.
Anatomisches Institut der Universität
Basel
Pestalozzistrasse 20
Basle Switzerland

SERR, Dr D M.
6 Mexborough Street
Leeds 7

SIEBENMANN Dr R. E.
Pathologisches Institut,
Schmelzbergstrasse 12
Zürich 6 Switzerland.

SLATER, Dr E.
Institute of Psychiatry
Maudsley Hospital
London, S.E 5

SLIZYNSKI, Dr B M.
Institute of Animal Genetics
West Mains Road
Edinburgh 9.

SMITH, Dr D Robertson
Dept. of Clinical Pathology
King's College Hospital Medical School
London S E.5.

STEWART Dr J S. S
Eastern District Hospital
Glasgow E 1

SWYER, Dr G I M
University College Hospital
London W C

TAVARES, Dr A. S.
Laboratorio De Anatomia Patologica,
Porto Portugal.

WIEDEMANN Prof H R.
Städtische Krankenanstalten
Kinderklinik,
Krefeld Germany

A BRIEF HISTORICAL INTRODUCTION

MURRAY L. BARR, M.D. F.R.S.C.
Professor and Head,
Department of Microscopic Anatomy
Faculty of Medicine,
University of Western Ontario
London, Canada

MY first duty and it is a pleasant one, is to thank those scientists of Great Britain who had the foresight to conceive this Symposium and the initiative and energy to carry their idea to the stage of fruition. We all regret that certain practical considerations made it necessary to restrict invitations with only a few exceptions, to scientists who would not have to travel long distances. It is hoped that our deliberations will be helpful to all who are interested in the problem of developmental sex anomalies and in certain aspects of cytology whatever their geographical location may be.

To cover the history of chromosomal sex and sex anomalies is obviously an impossible task in a short introductory comment. For the sex chromosomes alone one would have to start with the observation of the German zoologist, Henking in 1891 that in certain insects the chromosome number differs between males and females. It would then be necessary to describe the fundamental studies of the sex chromosomes by the American cytologists, McClung and Wilson, in the early part of the present century and the subsequent work of Painter and others on the sex chromosomes of man and other mammals. The history of sex anomalies would lead us even farther afield where special prominence would be given the now-classical work of Young of Baltimore, whose book on genital abnormalities (1937) is a landmark in the clinical study of sex anomalies. Instead of attempting the impossible I must be content to relate a few facts that concern the recognition of a sexual dimorphism in the intermitotic nuclei of mammals. This does not imply any feeling on my part, or that of my co-workers, that this recent development is any more important than the vast number of other contributions to the study of chromosomal sex and sex anomalies. The recognition of a sexual characteristic in resting nuclei is simply another in a long series of developments it has served the useful purpose of stimulating interest in this particular field of

research and was partly responsible for bringing this group together

As so often happens in scientific work, the first intimation that there is an imprint of sex in the resting nuclei of mammalian tissues came from an incidental observation while we were engaged on an entirely unrelated project. While serving with Medical Boards of the Royal Canadian Air Force conversations with Squadron Medical Officers and others turned at times to the problem of fatigue in aircrew On my return to academic work in 1945 there arose the question of a suitable line of research. The recollection of these conversations induced me to look into the complex problem of "fatigue by the only methods I knew in view of previous training and experience i.e. by using the techniques of neurocytology The investigation was supported by a grant from the Institute of Aviation Medicine, R.C.A.F Many studies of the effect of altered levels of activity on neuronal structure had been made since Hodge around 1890 described structural changes in the nerve cells of bees and swallows following busy periods of work and flight Even in recent times, the experiments involved the interaction of neuronal systems and synaptic transmission and, in order to simplify the experimental milieu it was decided to test the effect of antidromic stimulation of motor neurones. It was my good fortune at this time to have Ewart G Bertram a graduate in Science of the University of Western Ontario apply for admission to the department as a graduate student With the background just mentioned, we stimulated the hypoglossal nerve of the cat, allowing the animals to survive for different lengths of time before examining the neurones of the hypoglossal nucleus in sections stained with cresyl violet. There were chromatolytic changes on the stimulated side (Barr and Bertram 1951) but we still do not know whether these changes were caused by increased neuronal activity or whether they were a mild form of axon reaction following injury to the nerve fibres by the stimulating current. This question might have been resolved had our work not been directed into other channels by the following events.

While studying the altered neurones, it was noted that a rather conspicuous mass of chromatin tended to move from its usual position next the nucleolus for varying distances towards the nuclear membrane during the period of the mild dissolution of the Nissl bodies, and that the normal position of this mass of chromatin was restored when the cell recovered from chromatolysis It was decided to chart the movement of the chromatin mass in some detail since such a phenomenon is unusual in resting

nuclei. As the series of animals came under review it became apparent that the chromatin mass in question was present in the cells of some of them but not in others. Various possible explanations of this inconsistency had to be discarded, until our re-examination of the data on each animal, for which Dr Bertram had meticulously recorded the sex even though this had no bearing on the problem we had set out to study showed that the mass of chromatin which is known now as the sex chromatin was present in the hypoglossal neurones of females but not in those of males. This correlation led us into a new field of research and the end is not yet in sight. The foregoing account will answer a question that has been put to me many times 'Why was the Institute of Aviation Medicine R.C.A.F referred to in your first account of sexual dimorphism in resting nuclei?" (Barr and Bertram, 1949)

I wish at this point to acknowledge the invaluable assistance that was given by the graduate students and research assistants who participated in the ensuing programme of research in the department. At the risk of being unfair since all contributed valuable observations, three of them must be given special mention The major contribution of Dr Ewart G Bertram in the early and critical phases of the work has been related Dr Bertram continued his neurocytological studies in the United States and is now an Assistant Professor of Anatomy in Marquette University Milwaukee. Mrs Margaret A. Graham, who has been promoted to the role of housewife, carried out meticulous studies on the nuclear morphology of various tissues in the cat that included particularly valuable work on embryonal tissues. Dr Keith L Moore, now Assistant Professor of Anatomy in the University of Manitoba, was primarily responsible for the analysis of nuclear structure according to sex, in the nervous system of various mammals, in human tissues generally and in benign and malignant tumours of man. To these and the others as well as to two expert technical assistants, Mr J E. Walker and Mr C E. Jarvis, I am deeply grateful.

This fragment of scientific history may be of some slight interest to the particular group assembled here. In any event, I hope that I may be forgiven for reminiscing

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CYTOLOGICAL AND GENETIC ASPECTS OF NUCLEAR SEX

CHAIRMAN

PROF L S PENROSE

CYTOLOGICAL OBSERVATIONS RELATED TO NUCLEAR SEX

B M SLIZYNSKI Ph.D
Member of Scientific Staff,
Medical Research Council,
Institute of Animal Genetics,
University of Edinburgh

According to the classical view of textbook cytology the sex of the zygote, in the vast majority of animals, is determined at the time of fertilization by an appropriate chromosomal mechanism. In this the sex chromosomes, X and Y play a decisive role. In organisms in which the sex chromosomes differ morphologically the presence of a heteromorphic sex chromosome can be established as a rule in all cells by studying cell division. Recently it has been demonstrated that stainable bodies in the resting nuclei (sex chromatin nucleolar satellites, or drumsticks) can be used as a criterion for nuclear sex.

The most interesting fact of the whole problem of nuclear sex is the proportion of cells in which these stainable bodies can be recorded. These bodies are not present in all the nuclei of a female organism and they are not absent from all the nuclei of a male organism. The proportion in which they are found by various authors is usually far from that expected on a chromosomal basis, for all the cells of an organism are supposed to be of the same genetical constitution.

Two other facts should be pointed out in this connection one is the absence of a regular ratio between proportions for females and those for males and the other the great variation of the proportion from tissue to tissue For instance (Fig 1) variation in the proportion of cells containing sex chromatin in the skin of males covers the whole range of variation of the satellite body ("drumstick") in the blood cells of both males and females.

Since the nucleus of a cell contains the chromosomes it is natural to look for some interrelation between the chromosomes, particularly the sex chromosomes, and the nuclear sex. It is thus worth while examining the possibilities to see whether the remarkable proportions of cells with nuclear sex bodies could depend on some chromosomal mechanism.

The first hypothesis to be discussed is that of somatic segregation, whereby a cell of a male containing the sex chromosomes XY could produce two daughter cells of which one contains XX the other YY. However, although this hypothesis could explain the unexpected appearance of sex chromatin in some male cells,

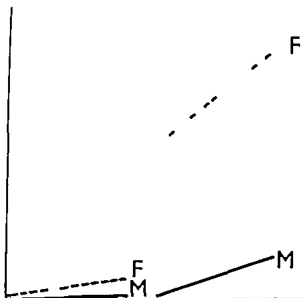


FIG. Sex ratio and variation from tissue to tissue of the nuclear sex. On the left-hand side the satellite body in blood cells (after Davidson and Smith); on the right-hand side the sex chromatin in human skin (after Moore, Graham, and Barr). F in females, M in males. Variation of proportion of cells containing sex chromatin in the skin of males covers the range of variation of the satellite body in the blood cells of both males and females.

it is useless since it cannot account for the absence of sex chromatin in some female cells.

The second hypothesis is concerned with a change of chromosome number. The constancy of chromosome number in the cells of man has been questioned (see review by Beatty (1954)), for Timonen (1950) and Therman and Timonen (1951) found great variations in the chromosome number in certain human tissues and considered that an aneuploid number of chromosomes prevailed in all the tissues studied. They leave open the question of the mechanism responsible for such variations. Timonen's findings have been questioned by other workers and are not generally accepted. However, somatic numerical chromosome

variation may be produced also by somatic polyploidy which seems to have had a somewhat better reception among cytologists. For the purpose of the present discussion it is of no consequence whether the additional X chromosomes in a cell come about by somatic variation connected with aneuploidy or with polyploidy.

Tentative application of the hypothesis of somatic numerical variation or more exactly of aneuploidy is discussed below. It

TABLE I
APPLICATION OF TIMONEN'S DATA TO NUCLEAR SEX

Timonen Data		Deduced No. of X chromosomes	Distribution of X Chromosomes among Female Cells						Analogy for Males			
No. of chromosomes	No. of cells		O	X	2X	3X	4X	5X	O	X	2X	3X
5	3					—	—	—			—	—
		4	6	4	—	—	—	—	8		—	—
5	6		6		—	—	—	—		5	—	—
10	70	6	9	8	—	—	—	—	40	30	—	—
15	176	300	—	252	24	—	—	—	56	50	—	—
30	60	209	—	1	49	—	—	—	56	04	—	—
35	147	244	—	70	77	—	—	—	35		—	—
40	5	000	—	30	65	—	—	—	5	00	—	—
45	46	90	—		44	—	—	—		43	—	—
50	100	2	—	—	65	7	—	—	—	90	8	—
55	9	22	—	—	5	4	—	—	—	7		—
60	7	8	—	—	3	4	—	—	—	3		—
65	6	7	—	—		5	—	—	—	4		—
70	4	1	—	—	—	4	—	—	—			—
75	5	16	—	—	—	4		—	—		1	—
80	6		—	—	—	3	3	—	—	1	4	—
85	4	5	—	—	—		3	—	—		3	—
90	4	6	—	—	—	—	4	—	—	—	4	—
95	5		—	—	—	—	4		—	—	5	—
00	7	30	—	—	—	—	5		—	—	6	
	1000	504			37	41	20	5			4	

is based on Timonen's data (1950) on one thousand cells from human uterine epithelium in the proliferative stage. The chromosome number varies from four up to one hundred per cell. The data of Timonen can be worked out to show that some female cells may not contain two X chromosomes and that vice versa some male cells may contain more than one X chromosome.

The distribution shown in Tabl I has been arranged on the assumption that the X chromosome has the same chance of being included in an aneuploid cell as any other chromosome. The available X chromosomes have been allocated by giving to each

cell one X chromosome, then following the same procedure for the second and subsequent X chromosomes until all the cells and X chromosomes have been used up By this method a distribution has been obtained with the smallest number of X chromosomes per cell. Any other distribution will give a larger proportion of cells with more X chromosomes. The analogy for males is based on the same principle, but the number of available X chromosomes has been halved since half the sex chromosomes are Y chromosomes in the male.

If the appearance of sex chromatin in a resting nucleus depends on the presence of two or more X chromosomes then the hypothesis of somatic aneuploidy could explain the proportions of nuclei with stainable bodies. This hypothesis also permits variation of the proportion of cells with sex chromatin when the distribution of chromosomes among the nuclei is altered

It is evident from the table that 436 about 44 per cent, of female cells and 42 about 4 per cent of male cells contain two or more X chromosomes. If three or more X chromosomes are required to produce the appearance of sex chromatin in the cell the proportions from the table will become 65 cells, 6 5 per cent for females and 1 cell, 0 1 per cent, for males.

A similar hypothesis assuming somatic polyploidy could be applied to nuclear sex. It will require the assumption that tetraploid nuclei in females and octoploid nuclei in males (both giving the same number of X chromosomes per nucleus) exhibit the sex chromatin. The frequency of these polyploid nuclei may directly correspond to the frequency of sex chromatin.

The hypothesis of somatic numerical variation (if in itself correct) may cover adequately most of the facts known about nuclear sex. There are however still some unexpected difficulties, for instance the fact that Purkinje cells in human brain have the same frequency of sex chromatin in males and in females. It may be that in this case the presence of one X chromosome is sufficient for the sex chromatin to appear This suggests that metabolic processes peculiar to particular tissues do play an important rôle in the phenomenon of nuclear sex.

On the border of chromosomal and metabolic hypothesis of nuclear sex stands the third hypothesis that the sex chromatin represents the so-called extra-chromosomal chromatin Tobias (1956) described how in the Gerbill during spermatogenesis some parts of the chromatin material become thrown off bodily from certain segments of the chromosomes and remain in the nucleus as a mass of chromatin not attached to any chromosome.

It is not known whether this extra-chromosomal chromatin can

be produced in somatic cells and how it would behave during subsequent cell divisions. However it may be worth while to keep it in mind as one of the possibilities pertaining to the nuclear sex. In this connection it is interesting that in a recent publication in *Nature* (1957) Ashley postulates that sex chromatin must be regarded as a sex characteristic rather than as belonging to the XX chromosomes.

The fourth hypothesis is connected with chromosome movements. The classical study by Barr Bertram and Lindsay (1950) on the nuclear sex in nerve cells of the cat gives the proportion of cells showing the nucleolar satellite for females 56 to 87 per cent

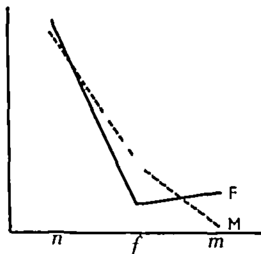


Fig. 2. Position of nucleolar satellite in cat nerve cells in females (F) in males (M), the nucleolus ( ) free in the karyoplasm (*f*) and at the nuclear membrane (*m*) From data of Barr Bertram and Lindsay (950)

and for males 2 to 6 per cent. They noticed also a very interesting fact, namely that the nucleolar satellite can be at the surface of the nucleolus, at the nuclear membrane or free in the nuclear space. The proportions of male and female cells with a nucleolar satellite in any of these three positions is presented in a diagram (Fig 2) There is a statistically significant frequency difference between the sexes and it is also clear that the position of the nucleolar satellite depends on the sex of the cell.

It is probable that the variation of the position of the satellite represents the movements to which it is subjected. These can be compared with movements of the polytene chromosomes in

salivary gland cells of dipteran larvae. In the larval salivary gland of *Simulium* the polytene chromosomes occupy different positions which can be arranged in a sequence (Fig 3 Nos. 1–5) In certain cells thin chromosomes are evenly distributed throughout the nucleus with the nucleolus visible in the centre The next stage is characterised by the withdrawal of the chromosomes from the periphery of the nucleus and their subsequent gathering in the central parts leaving empty most of the outer sphere of the nuclear vesicle The nucleolus becomes invisible probably being overshadowed by the chromosomes. Later on the chromosomes expand again and occupy the whole of the nucleus, being much thicker than before. Chromosome movements are as a rule not synchronous in all cells. Similar behaviour of chromosomes in *Drosophila virilis* has been described by Bodenstein (1943)

Movements of salivary gland chromosomes of dipteran larvae differ from the suggested movements of resting stage chromosomes of mammalian cells mainly in the fact that the latter possibly do not involve any internal multiplication of chromosome strands, but are most probably connected with other types of nuclear/cytoplasmic exchanges. Salivary gland chromosomes remain longer at the nuclear membrane than at the centre of the nucleus, while mammalian sex chromatin spends more time at the surface of the nucleolus. It is possible that the appearance of sex chromatin represents one phase in chromosomal movements and that the rhythm of these movements is not the same in the two sexes. Such an explanation does not involve any somatic variation of chromosome number and can be accepted as a working hypothesis of the mechanism of nuclear sex.

Variation in size and in intensity of staining of the chromosomes can be related to the nuclear sex. According to the prevailing opinion the size of the chromosomes is under genetic control and remains constant in all cells of the organism. However in mouse spermatogenesis it has been observed that in some cells the chromosomes are exceedingly small (Fig 3 Nos. 6–9) The nucleus is reduced in size and so is the cell body Changes in the intensity of staining have also been recorded in mouse spermatogenesis. Pale staining metaphase plates alongside well stained ones (Fig 3, No 10) can be quoted as an example of such changes in the intensity Three grades of staining from normal to extreme pallor are demonstrable It is suggestive that Barr Bertram and Lindsay found that in cat nerve cells the intensity of staining of the sex chromatin is also variable

These variations in size and staining reaction occur in the translocation mouse stock, *translocation No. 6 (Slizynski 1957) They*

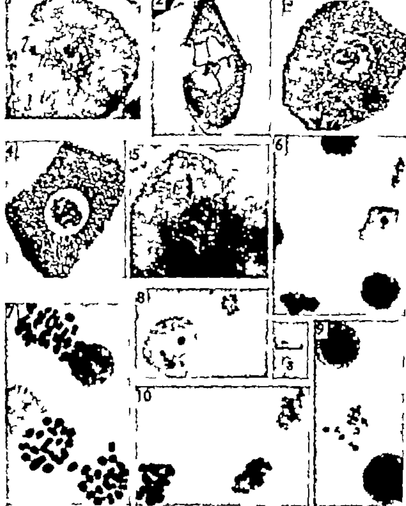


Fig. 3 Nos. –5. Movement of chromosomes in the nuclei of cells from larval salivary gland of Simulium sp.

Nos. 6– 0. Chromosomes from mouse spermatogenesis.

No. 6. Side view of two first meiotic metaphase plates to show the difference in size of the chromosomes.

No. 7 Two first meiotic metaphase plates for comparison with Fig. 8 the top, late diakinesis for comparison with Fig. 9.

No. 8. Polar view of the first meiotic metaphase plate of cell with small chromosomes.

No. 9. Late diakinesis of cell with small chromosomes.

No. 0. Three first meiotic metaphase plates in side view to show the differences in intensity of staining.

s. Represents the scale of magnification which is equal to 42 microns for Nos. –5 and 7 microns for the *remaining* figures.

occur in animals heterozygous for this translocation in combination with any of the four other mouse translocations (Nos. 5 83, 264 and 281) but not in the homozygous condition or in crosses with any of the six other translocation stocks (Nos S 2 7 8 138 and 190) although they may have been present in very low frequency It should be stressed that both changes form a graded series in all cases and thus cannot be attributed to the loss of a chromosome or to the mutation The best explanation seems to be the interaction of a certain chromosomal constitution with metabolic activities inside the nucleus, illustrating how a profound morphological effect can be produced by metabolic causes.

Summing up it can be concluded that of the four hypotheses two seem possible. The frequency of the sex chromatin could be explained by the hypothesis of somatic variation of the chromosome number assuming that that hypothesis itself is based on fact. The other explanation is that of chromosome movements with different rhythms for the two sexes This suggests that the nuclear sex, although founded principally on a chromosomal mechanism represents the morphological end result of metabolic processes

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DISCUSSION

FORD Dr Slizynski's hypothesis is, of course a formal possibility but if it applies to human endometrium it must apply to other tissues too and not only in man but in other organisms It is the experience of chromosome cytologists that as the techniques for examining the somatic chromosomes have been improved, and I think they have been improved very considerably over the last four or five years the evidence for this somatic variation in number somatic aneuploidy as Dr Slizynski called it, gets less and less. Now from my own experience in a whole series of mammals—about 10 altogether—but particularly in the bone marrow spleen lymph nodes and embryonic tissue in the mouse

and threw. I agree that there is an *asymmetrical distribution* of chromosome numbers about the standard for the species but the numbers of cells with chromosomes greater than the standard number for the species is very low indeed, perhaps two in a thousand. On the other side there is a long tail going down to numbers perhaps even approaching the haploid number. The extent of this tail varies from preparation to preparation. In the better ones it is very small indeed. I consider this tail to be almost wholly an artefact due to the fact that as we make these preparations by squashing we break some of the cells and lose some of the chromosomes, and I think the reports of excessive variation in chromosome number can be attributed to that fact. There are two other small pieces of evidence in favour of this. If a somatic cell can stand the deficiency of one chromosome it can surely stand the deficiency of half that chromosome, but from irradiation experiments, which are still unpublished by my former colleague Sharman there is evidence that a lesser deficiency that is less than a whole chromosome, does not appear. Secondly there is genetic evidence that if this somatic aneuploidy occurred in animals heterozygous for a skin pigmentation gene we should find localised areas of the recessive type.

SACHS. Just two small comments. First of all I would like to reinforce what Dr Ford said. In some examinations I made on human material a few years ago I was quite unable to substantiate the work of Therman and Timonen and I fully agree with him that the idea and even the facts of chromosomic inconstancy are extremely dubious. Secondly I think it is hardly necessary to invoke them to explain the differences in the proportion of nuclei with and without the chromocentre or sex chromatin or whatever you like to call it. In analysis limited to the old spinous cells of the skin we found about 95 per cent of the cells with a chromocentre in females, and that is in sections, where you would expect to get a few lost in any case. I think even trying to apply this rather dubious hypothesis of Timonen to the so-called ratios which one gets in the proportion of nuclear sex is quite unnecessary because no accessory hypothesis is really needed in order to explain the observed facts.

SEER. I would also like to substantiate what Dr Sachs and Dr Ford have just said. We tried to cut out any damage to the tissues in preparation and to obtain a pure tissue by culturing amnion cells to see if we could get a higher percentage, something approaching 100 per cent. In fact in the pure culture in which we allowed the cells to settle on a plate, we had counts of 96–97 per cent. We are now attempting to do this on cultures from one

single cell from which we hope will be able to produce something approaching 100 per cent.

C. LEUCHTENBERGER I too want to support Dr Ford and Dr Sachs with completely independent data not on chromosomal counting but on DNA determinations which we did in the endometrium to test the data of Timonen. We found that there is a very good correlation between chromosomal number and DNA content per nucleus. We found a rather good diploid peak in the endometrium with a very few cells which were hypodiploid and a very few in a small tail, which were towards the tetraploid state. I think that even these two tails which we found by DNA may be explained on the basis of the method which we used. As a rule there was a very good peak, that is a wonderful correlation between the diploid number of chromosomes and the DNA content.

KLINGER Again, I would like to confirm in amnion we too were able to get counts up to 99 per cent of sex chromatin positive cells in culture preparations when no sections were made On the average, however this was reduced to 91–92 per cent. There is definitely a small percentage of nuclei which have no sex chromatin and perhaps in these the extremes of chromosomal variations may come into play On the whole one does have very much higher counts on material not sectioned than on sectioned material.

SLIZYNSKI In my talk I presented all hypotheses impartially and did not particularly favour the hypothesis of somatic aneuploidy of Timonen.

2

HUMAN CHROMOSOMES

C. E. FORD Ph.D

Member of Scientific Staff,
Medical Research Council,
Radiobiological Research Unit,
Atomic Energy Research Establishment,
Harwell, Berkshire, England

Two years ago everyone knew that human beings had 48 chromosomes. A year ago everyone knew that the number was 46. Now the claim comes from Japan that both these numbers occur in different individuals, as well as the intermediate number 47. What is the evidence, and what are we to believe?

The problem is best treated historically. The earlier investigators all used variations of the classical histological procedure of fixation, embedding, sectioning and staining. Observations were made at metaphase, either of mitosis (usually in spermatogonia) or of meiosis in spermatocytes. But notwithstanding the careful attention to details of technique the chromosomes were closely crowded together in the equatorial plane of the spindle so that counting was at best tedious and difficult and at worst quite impossible. In this early period the names which stand out are those of de Winiwarter and Painter. De Winiwarter's classical paper, published in 1912, ended the initial phase of wildly inaccurate counts. He said that the diploid number in men was 47 and that there was an unpaired X chromosome in primary spermatocytes. Painter (1923) followed with the assertion that 48 was the correct number in both sexes (although in a preliminary note he had been uncertain whether it should be 48 or 46). Thereafter it seemed that the single remaining point at issue was whether the male had 47 chromosomes (XO) or 48 (XY). Only de Winiwarter and his associates supported the XO interpretation, and since decisive evidence of the existence of a Y chromosome in primary spermatocytes was given by several independent observers, the figure 48 gained the day and came to be widely quoted in texts of all sorts.

Work on mammalian chromosomes was slow to get going after the Second World War, but when it did a technical revolution followed. In rapid succession a number of innovations was

introduced to improve the accuracy and speed of observation by increasing the separation between individual chromosomes. They included the squash method (originally developed by Heitz for plant material in the thirties) the use of colchicine to inhibit spindle formation, and the exposure of tissue to a hypotonic solution before fixation in order to swell the cells and scatter the chromosomes within them. At the same time the sharpness and specificity of staining were improved by the adoption of the Feulgen and acetic-orcein methods. Each of these steps alone makes a considerable difference to the ease and reliability of counting and the effect of combining them can be enormous. *Preparations are also obtained much more rapidly* In the simple squash method, freshly fixed tissue is bulk stained and then teased or tapped out in a drop of 45 per cent acetic acid on a standard glass microscope slide before covering and squashing by thumb pressure between sheets of filter paper It may be thought that such a procedure would damage the chromosomes. Their spatial relationships are necessarily altered, but, when properly fixed, they are resistant structures and will stand up to a considerable amount of apparent ill treatment without breakage or morphological change. Squashing is, however liable to rupture the cytoplasm of some cells, so that fragments carrying one or more chromosomes may be lost and counts of less than the standard number result. Although the principles behind these newer methods are well understood it is not to be supposed that they have yet been thoroughly exploited there is plenty of opportunity for further advance

In the summer of 1956 everyone was surprised (to say the least) to learn that the accepted human diploid number of 48 might not be correct after all. Taking advantage of the new methods Tjio and Levan (1956) counted 46 chromosomes consistently in dividing cells of tissue cultures established from four aborted human embryos. The cultures were primary explants of lung which had been grown for a few days only and the quality of the photographs which illustrated their paper was sufficient guarantee of the accuracy of their counting It is known that changed chromosome numbers commonly appear in cultures of normal tissue after several serial transfers (Hsu and Moorhead, 1957) and it is possible that disjunctional irregularities are more common at mitosis in primary cultures than in intact tissue But the constancy of the counts made by Tjio and Levan was such that it could be safely inferred that there were 46 chromosomes in the fœtal lung cells from which their cultures were established. Later in the same year the new number was confirmed in preparations

made from testicular tissue taken from three men 46 chromosomes being found at spermatogonial metaphase and 23 bivalents at metaphase in primary spermatocytes (Ford and Hamerton, 1956)

The difference between the new and the old counts had to be explained somehow The possible existence of a real variation in chromosome number between individuals was considered to be very unlikely all the circumstances pointed to a persistent error in counting as much the more likely explanation (Ford and Hamerton 1956) However very recently it has been claimed that in a sample of twenty-one Japanese men, sixteen had 48 chromosomes, one of them 47 and four 46 The observations were made on testicular preparations by Kodani (1957) who believes that the additional chromosomes (in excess of 46) may be akin to the supernumerary genetically inert, chromosomes known to occur in many plants and several species of invertebrates. In these species the frequency of supernumerary chromosomes varies from one population to another but within a population the distribution is invariably unimodal (except in some species of grasses where a special mechanism is known to operate) Kodani's sample is so sharply bimodal as to suggest strongly that it was drawn from a population which does not conform to this rule A variety of mechanisms could be postulated to account for such a departure, ranging from controlled non-disjunction in meiosis to assortative mating and several of them should be capable of demonstration by cytogenetic methods if they occur Should Kodani's counts and interpretation be confirmed it would be a situation without known parallel among vertebrates.

Meanwhile Dr J H Tjio informs me that he has examined cultures from seven more embryos and that all have 46 chromosomes. Mr J L Hamerton and Dr U Mittwoch and myself independently have made counts on testicular and bone marrow preparations from nine more individuals with the same result. Finally Dr T C. Hsu, Dr J G Gall, and Dr E A. McCullogh have each kindly allowed me to quote their independent unpublished (and in the last case preliminary) observations on human tissue cultures. Most of these were freshly established and they included one series grown from various organs of a female negro embryo The observations were made in three different laboratories in the United States and Canada, and all support the number 46 It seems reasonable to conclude that persons with more than 46 chromosomes if they occur at all must be relatively rare among white people Further counts on individuals of the Japanese and other races will be of great interest.

The precise human chromosome number is not directly relevant to the problems with which this Symposium is concerned, but it has been discussed at some length to give an idea of the present state of development of human cytogenetics. The identification of the sex chromosomes is of more immediate importance. At diakinesis and metaphase of first spermatocytes, the X and Y chromosomes are usually associated terminally to form a prominently unequal and asymmetrical bivalent, and are accordingly easily recognized (*Fig* 1) The X chromosome is the larger and is frequently bent in the middle at a position which probably represents the centromere The Y chromosome is much smaller It is by no means uncommon at late diakinesis and metaphase to find that X and Y are separate (they have probably disjoined precociously) When this occurs Y tends to become round, and X shorter thicker and more rod like (Fig 2) Although recognition is so straightforward in male meiosis the sex chromosomes have not yet been identified at mitosis. Here the chromosomes look very different, especially after colchicine or Colcemid treatment (Fig 3) The X must be one of the medium-length chromosomes and may prove impossible to distinguish. On the other hand there would seem to be a reasonable possibility of being able to recognize the Y as one of the shortest chromosomes present, particularly if, as seems likely it has a terminal centromere

There is now strong support for the belief that the prominent chromatin body or chromocentre, present in the interphase nuclei of many tissues of females is the product of fusion of two heterochromatic regions one in each X chromosome The correlation of this structure with female sex in normal individuals of several mammalian species is so complete that its presence or absence is now widely accepted as a reliable indicator of sex. The possibility that it is in some way a secondary product of the female genotype, perhaps indicative of female rather than male hormonal status would seem to have been eliminated by the fact that cat embryos can be classified as chromatin-positive or chromatin negative at stages before any morphological signs of sex differentiation have appeared (Graham, 1954) But although the present evidence indicates that the presence of two X chromosomes is *necessary* for the appearance of 'sex chromatin, its characteristic absence in certain tissues of females (e g the basal layer of the skin) shows that the XX constitution alone is not *sufficient* some other unidentified physiological factor is required for its expression By hypothesis, failure of this factor would lead to the non-appearance of the sex chromatin in an

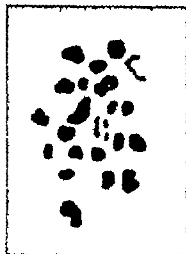


FIG. First spermatocyte metaphase from testis tubule exposed to hypotonic saline 19 bivalents. The unequal XY bivalent is top right. (× 2900)

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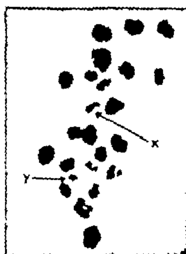


FIG. First spermatocyte metaphase from testis tubule exposed to hypotonic saline. 19 bivalents plus univalent X and univalent Y (× 2900.)

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XX individual. The complementary possibility of the spurious appearance of 'sex' chromatin in a genetic male is more difficult to envisage and there is no reason to suppose that it might occur. The obvious need is to make combined observations of both the 'sex' chromatin and the sex chromosomes in tissues where the physiological conditions are abnormal. Excellent material for such a purpose would be provided by human intersexual individuals. Teratomata should also provide valuable information. The direct determination of the sex chromosome constitution in such material would seem to be a more than formidable undertaking when the normal number of chromosomes is still a matter of debate and the X and Y chromosomes have not been identified in somatic mitosis. Nevertheless, it should be possible for a limited contribution to be made now and I am confident that the continuous improvement of technical methods will steadily increase the range of situations which could be studied.

At the present time the best opportunity would appear to be offered by the chromatin positive cases of Klinefelter's Syndrome. In some of these patients spermatogenesis occurs, although only sporadically in testes which are appreciably reduced in size (Ferguson-Smith *et al.*, 1957). Current theory would predict that a symmetrical XX bivalent should replace the asymmetrical XY bivalent at diakinesis and metaphase in primary spermatocytes. This should be capable of verification by existing technical methods, although it might require a lengthy search before a tubule with spermatocytes in the required stage was found.

The intriguing explanations of human intersexual states put forward recently by Danon and Sachs (1957) suggest the possibility of a cytogenetical check in two other series of cases. They interpret cases showing the testicular feminization syndrome as having an XXY sex chromosome constitution. These patients should therefore have one additional chromosome in their somatic cells. In the absence of spermatogenesis, dividing cells would be required from some other part of the body. The short term bone marrow culture technique developed by Lajtha (1952) provides satisfactory material for chromosome number determinations, although the use of cells cultured from material taken at a biopsy which caused less discomfort to the patient would no doubt be preferable. The second series of cases which would be of interest to investigate by these methods is the gonadal agenesis group, some of which Danon and Sachs suggest may be XY/XX or XX/XO mosaics.

Although it is doubtful whether direct observations of human chromosomes will ever prove to have clinical value if they can

help to establish unequivocally the relationship between the sex chromatin and the sex chromosomes they will have performed a useful service for clinical medicine.

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DISCUSSION

LENNOX I wonder if we could ask Dr Ford to lay down what we ought to do with a testicular biopsy remembering we have to do both histology and a chromosome count?

FORD We usually put the small piece of testicular tissue first of all into hypotonic saline (half normal physiological saline) for half an hour Then fix in acetic alcohol for 1–2 hours, and stain by the Feulgen method details of that you can find of course in Darlington and La Cour s little book. The material can be stored in 45 per cent acetic acid in the deep freeze for a long, long time and be referred to any time. Recently I have found that citrate is rather better than hypotonic saline. We have only had five cases so far and this chromosomal cytology technique is highly empirical and I am sure we will get progressively more efficient methods.

SACHS I am particularly keen to know whether there is any thing in the hypotheses of an XXY and possibly of an XO which I was once rash enough to put forward. If any clinician has a case which is of a peculiar nature it is worth while examining the chromosomal complement using the methods as detailed by Dr Ford.

BARR In many sex anomalies testicular tissue is not available either because gonads are not present at all or are in the pelvis and not available. Do you think it worth while exploring the possibility of growing fibroblasts, say from dermis, in tissue culture and using that sort of approach so that it can apply to all types of intersex?

FORD Yes most certainly that is what I implied, but that would involve more work.

3

THE SEX CHROMATIN BODY ITS FINER STRUCTURE AND BEHAVIOUR DURING AMITOSIS OR ENDOMITOSIS

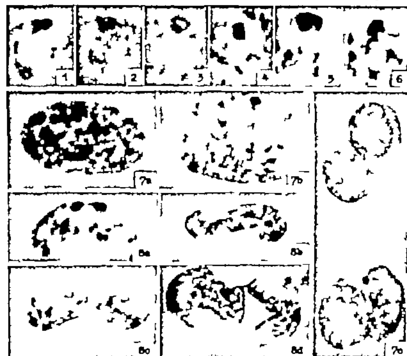
HAROLD P KLINGER

Department of Anatomy
University of Basel, Switzerland

BARR and his co-workers (Barr *et al.*, 1950 Graham and Barr 1952 Moore and Barr 1953) proposed that the sex chromatin of the female nucleus is the result of the fusion of the heterochromatic portions of the two X chromosomes Crouch and Barr (1954) found that some sex chromatin bodies consisted of two portions and suggested the term diplococcus for such split sex chromatin (Figs. 1 and 2) This diplococcus form should not be confused with the double form which I have described briefly elsewhere (Klinger 1957) and which I will discuss below

This study was undertaken with the intent of determining the frequency with which the diplococcus sex chromatin occurs in various human tissues Details concerning the materials and methods as well as the complete findings are given in a forth coming publication (Klinger 1958) With the aid of an improved staining procedure (Klinger and Ludwig 1957) as well as with the phase contrast microscope it was possible to recognize the diplococcus form of the sex chromatin in all of the human tissues studied. In some tissues the diplococcus is more difficult to recognize than in others. In part this is due to technical factors. The thin embryonic membranes which can be mounted whole and fixed rapidly give the highest counts of the diplococcus sex chromatin In these membranes 13 5 per cent of the sex chromatin is of the split form. Errors due to the clumping action of the fixative and the malalignment of the sex chromatin in relation to the optical axis probably reduce the frequency with which two such small bodies, which are about 0 5 μ in diameter and separated by a distance of only 0·3 μ, can be resolved. It is therefore very likely that all sex chromatin consists of two portions. In addition, other details of finer structure can be recognized within the sex chromatin. Often vacuoles most commonly one or two (Figs. 3

and 4) sometimes up to seven, are present. The sex chromatin may show spiral forms, which when projected on to a single plane



FIGS. 1 to 6 Portions from amnion epithelium and chorion connective tissue nuclei showing the details of structure of the sex chromatin.

FIGS. 7a to c show the successive stages of reconstructed amitosis or endomitosis from amniotic epithelium. The nuclear pairs in each belong to a single cell

FIGS. 8a to d. The same process as in Fig. 7 but these are chorion connective tissue nuclei. Note that in d the daughter nuclei are still connected by a fine nuclear strand.

All nuclei are stained according to the thionin method described in (8) Figs. 3, 5, 7b and 8a are reproduced at a magnification of 3300 × all others at 2600 ×

For further explanations of the illustrations see the text.

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suggest the letters "M" or "S" (Figs. 5 6) Chromatin strands sometimes emerge from such structures (Fig 6) In preparations stained according to the Feulgen method with light green as a counter-stain, a small green nucleolus-like body was sometimes found adjacent to the red sex chromatin [see colour plate III Fig 1c in Klinger (1957)]

These findings seem to support the theory of Barr *et al.* stating that the sex chromatin is derived from the fusion of the two heterochromatic (heteropycnotic) portions of the X chromosomes. In this respect the work of Ohno *et al.* (1956) is of particular interest. These workers showed that the sex chromosomes were heteropycnotic from the pre-leptotene through the diplotene of the first meiotic prophase in the rat testis. "The X chromosome again becomes heteropycnotic at the telophase. Thus the daughter nuclei which have received the X chromosome are easily distinguishable from the others which have received the Y chromosome—in this material it has been found that the Y chromosome alone is too small to be recognized consistently if it be heterochromatic. Moore and Barr (1953) reported that the sex chromatin could not be identified in rat nuclei. Others (Castro *et al.* 1956 Klinger 1952) have been able to find nuclear sex differences in some tissues in this animal. It seems most likely therefore, that the heteropycnotic body Ohno *et al.* found in the spermatocyte is related to the sex chromatin in the somatic nuclei. It is thus possible that they have shown us the transition from the sex chromosomes (or at least X chromosome) of the dividing nucleus to the sex chromatin of the interphase nucleus. In their primary spermatocyte there was only one X chromosome to follow and yet it yielded a heteropycnotic mass which was large enough to be well visualized. It is reasonable to believe that two X chromosomes would yield a double mass of the diplococcus type. The pyroninophilic and the ribonuclease-digestible vesicle which the above cited workers found to envelop the heteropycnotic sex chromosomes may be related to the nucleolus-like structure which I often found in relation to the sex chromatin. The vesicle however disappears during diakinesis in the rat spermatocyte. It could possibly persist in the somatic nuclei and would help explain some of the described details of the internal structure of the sex chromatin. Similar studies to those of Ohno *et al.* on somatic nuclei during mitosis could possibly settle the problem of the direct origin of the sex chromatin of the female nucleus.

In addition some nuclei in amnion and chorion were seen to contain two complete sex chromatin bodies, each the same size as a single sex chromatin. Either or both could be of the diplococcus form (Figs. 7*b* 8*a–c*). These double bodies were separated by large distances. Petry and Damminger (1956) as well as others claim that amitosis is the means of cell division in amnion epithelium. They base their claim partly on the fact that they find no normal mitoses in these tissues. According to personal

observations this holds true only for the amnion of newborn infants. In earlier embryonic stages mitoses can be found with regularity A more detailed report on these observations is forthcoming It is interesting however that in the trophoblastic membranes from newborn infants nuclei could be found which show forms suggestive of amitosis or endomitosis. In such nuclei double sex chromatin bodies are often found From a reconstruction of the division cycle in fixed material (Figs. 7 8) it seems as if the sex chromatin enlarges while the nuclear chromatin becomes more abundant (Fig 7*a*) the sex chromatin as well as the nucleolus then divides and moves to the opposite poles of the nucleus which by this time has assumed a dumb-bell shape (Figs 8*b* and *c*) and finally the nucleus divides (Fig 8*d*) Each daughter nucleus receives one sex chromatin body and both nuclei remain within one cell In this way many cells result with two or more nuclei each nucleus containing a sex chromatin body (Fig 7*c*) It is too early to be able to evaluate these observations completely Interpretation of reconstructions of a nuclear cycle from fixed material can at best serve as a basis for a working hypothesis. Studies are now in progress in this laboratory which, it is hoped, will throw further light on this problem as well as the general problems of amitosis and endomitosis.*

(*The author is greatly indebted to Prof G Wolf Heidegger Director of the Department, for the support which made this work possible Thanks are due also to Prof Th Koller Director of the Women s Hospital of Basel for the provision of material*)

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Recently we found (Klinger H. P and Schwarzacher H. G (958) *Nature* 181 1150), that female nuclei which contain more than one sex chromatin mass were polyploid, and that with each doubling of the diploid DNA value an additional sex chromatin mass appeared. Male nuclei with sex chromatin-like bodies were all polyploid, but not all male polyploid nuclei had sex chromatin bodies.

These findings seem to support the theory of Barr *et al.* stating that the sex chromatin is derived from the fusion of the two heterochromatic (heteropycnotic) portions of the X chromosomes. In this respect the work of Ohno *et al.* (1956) is of particular interest. These workers showed that the sex chromosomes were heteropycnotic from the pre-leptotene through the diplotene of the first meiotic prophase in the rat testis. "The X chromosome again becomes heteropycnotic at the telophase. Thus the daughter nuclei which have received the X chromosome are easily distinguishable from the others which have received the Y chromosome—in this material it has been found that the Y chromosome alone is too small to be recognized consistently if it be heterochromatic." Moore and Barr (1953) reported that the sex chromatin could not be identified in rat nuclei. Others (Castro *et al.*, 1956; Klinger, 1952) have been able to find nuclear sex differences in some tissues in this animal. It seems most likely, therefore, that the heteropycnotic body Ohno *et al.* found in the spermatocyte is related to the sex chromatin in the somatic nuclei. It is thus possible that they have shown us the transition from the sex chromosomes (or at least X chromosome) of the dividing nucleus to the sex chromatin of the interphase nucleus. In their primary spermatocyte there was only one X chromosome to follow and yet it yielded a heteropycnotic mass which was large enough to be well visualized. It is reasonable to believe that two X chromosomes would yield a double mass of the diplococcus type. The pyroninophilic and the ribonuclease-digestible vesicle which the above cited workers found to envelop the heteropycnotic sex chromosomes may be related to the nucleolus-like structure which I often found in relation to the sex chromatin. The vesicle however disappears during diakinesis in the rat spermatocyte. It could possibly persist in the somatic nuclei and would help explain some of the described details of the internal structure of the sex chromatin. Similar studies to those of Ohno *et al.* on somatic nuclei during mitosis could possibly settle the problem of the direct origin of the sex chromatin of the female nucleus.

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4

MAMMALIAN SEX CHROMOSOMES

J. L. HAMERTON B.Sc., A.R.C.S.
British Museum (Natural History)
South Kensington, London, S.W.7

In mammals no exception has yet been reported to the rule that the male is the heterogametic sex, possessing two unlike sex chromosomes, a larger X and a smaller Y whereas the homogametic female has two identical X chromosomes. The unlike sex chromosomes of the male vary greatly in size both relative to one another and to the other members of the diploid complement, technically termed autosomes. The field vole *Microtus agrestis* for example has giant sex chromosomes, both the X and Y being far larger than any autosome. In another rodent, *Rattus natalensis* (*Mastomys coucha*) both sex chromosomes are again larger than any autosome though the disparity is not so striking as in *Microtus*. In primates generally and in many other rodents (for instance the mouse) the X is a medium-sized chromosome and the Y one of the smallest of the set. Finally in many marsupials, the Y is so reduced in size as to offer considerable difficulties in detection. As yet there is no unequivocal instance of an XO male in any mammalian species, though it may be remembered that for many years de Winiwarter and his followers contended that there was no Y chromosome in man. Two possible cases have been reported recently—Matthey (1957) has reported 17 chromosomes in the somatic cells of both sexes in the fossorial vole *Ellobius lutescens*. In another and closely related species *Microtus* (*Chilotus*) *oregoni* the same author (1957) has reported chromosome numbers of 17 in the male and 18 in the female suggesting the occurrence of an XO male in this species. A full interpretation of the sex determining mechanisms in both these species would be of great interest.

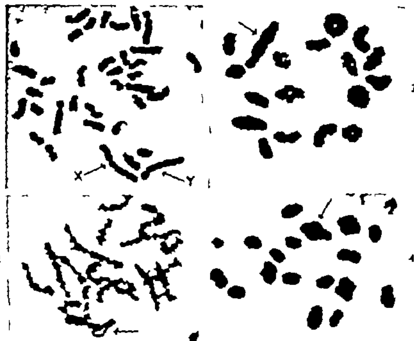
In a very few species a second, different, Y chromosome occurs, so that males regularly have one more chromosome than females. Some five instances of such multiple sex chromosome mechanisms have been reported in mammals, of which probably the best known are those of the marsupial *Potorus tridactylus*, and of the common shrew *Sorex araneus*. In both these species the second Y

chromosome (Y_2) is much larger than the original Y chromosome (Y_1). The biological significance of this type of sex-determining mechanism is obscure.

The behaviour of the sex chromosomes at the first division of meiosis has been the subject of controversy for a number of years. Koller, Darlington and their collaborators (1934–1941) interpreted the sex chromosomes as composed of homologous segments (which pair at the prophase of meiosis and form chiasmata) and heterologous, or differential, segments which do not pair. With the exception of the cat (Koller, 1941b) in which it was claimed that there was a median differential segment separating two distal pairing segments, the X and Y chromosomes of all species examined were supposed to consist of two segments, one of each type. If this hypothesis is correct, genes located in the pairing segments of the X and Y would presumably undergo crossing over with one another (and therefore with sex): they would thus be only partially sex linked, whereas genes located in the differential segments would be totally linked with sex. No certain examples of partial sex linkage in mammals have yet been reported. On the basis of this hypothesis the structure of the sex bivalent at diakinesis and metaphase is controlled by the position of the centromeres in relation to the pairing and differential segments and by the number, position and amount of movement of the chiasmata: terminal associations are interpreted as arising from the movement of previously interstitial chiasmata to the end, the process being known as terminalization (Darlington, 1937).

Makino (1941), Matthey (1949) and others consider, on the other hand, that the X and Y chromosomes remain as separate entities throughout meiosis and that therefore no crossing over can occur between them. According to this hypothesis the terminal association of the X and Y as seen in many mammals, including man, is interpreted as being due, not to terminalization of previously interstitial chiasmata, but to some more "perfunctory" association (Makino, 1941). According to Sachs (1954, 1955) at pachytene in the mouse and in man, the sex chromosomes are not paired in the same way as the autosomes but are included in a special "sex vesicle" and chiasmata are not formed between them. Slizynski (1955) however believes that the X and Y chromosomes in the mouse are in fact paired normally at pachytene, but unlike the autosomes are also associated terminally with a "puffy" region which is presumably equivalent to the "sex vesicle" of Sachs. In his 1954 paper Sachs goes on to say that "the absence of chiasma formation between the X and Y seems to be characteristic of all mammals which have so far been studied".

Although experience of the sex chromosome pair in man and the mouse (Ford and Hamerton 1956b and unpublished) is not inconsistent with Sachs' interpretation, its behaviour in *Rattus natalensis* (= *Mastomys coucha*) is more nearly in accord with the



Photomicrographs of the chromosomes of *Rattus* (*Mastomys*) *natalensis* (700.) Arrows point to the sex chromosomes. Fig. Spermatogonial mitosis. Fig. 2 Pachytene of spermatocyte meiosis sex bivalent showing precocious condensation. Fig. 3. Diakinesis of spermatocyte meiosis sex bivalent showing terminal association. Fig. 4. Diakinesis of spermatocyte meiosis sex bivalent showing subterminal association.

views of Koller and Darlington. Only a brief account of the sex chromosomes of this species has been published (Ford and Hamerton 1956a) As already stated the X and Y are the two largest chromosomes of the complement, X being a little larger than Y This is well shown at spermatogonial metaphase (Fig 1) No sex vesicle can be detected at pachytene instead there are three clearly defined regions—a terminal paired region, an intercalary unpaired (differential) region, and a second terminal strongly heteropycnotic paired region (Fig 2) The structure of the sex bivalent in this species appears to be similar to that

inferred by Koller (1941a) to exist in the cat. It may be noted in passing that at pachytene the sex bivalent is precociously condensed in relation to the autosomal bivalents. This is a common, and as yet unexplained property of the sex chromosome pair in mammalian *spermatocyte meiosis (cf. Slizynski, 1955, on* the mouse). Examination of the XY bivalent at diakinesis reveals two types of structure one in which there is undoubted terminal association (Fig 3) and another in which association by a classical subterminal chiasma seems to be equally clear (Fig 4). Whereas the first type can be interpreted according to the views of either Koller and Darlington or Makino Matthey and Sachs the latter can be explained only by the hypothesis of Koller and Darlington.

It is to be hoped that with advances in technique the interpretation of the morphology and behaviour of the 'sex vesicle' will be clarified. The structure has been observed at pachytene in a variety of species, and has been stated by Sachs (1954 1955) to contain in part or in whole the unpaired sex chromosomes. It is visible from the earliest stages of meiosis as a more or less heteropycnotic vesicular-shaped structure but disappears by late diplotene at which time the sex bivalent can generally be identified as a definite chromosomal body. A detailed review of the literature on the 'sex vesicle' in mammals is given by Tobias (1956).

Finally if a one to one relationship between cytological chiasmata and genetic crossing-over exists for the sex chromosomes as it is generally believed to do for the autosomes (Darlington, 1937) then although the genetic evidence is still inconclusive, the cytological evidence, as shown here is not incompatible with the occurrence of partial sex linkage in mammals.

(The author is indebted to Dr C E Ford of the M.R.C Radiobiological Research Unit at Harwell for many valuable suggestions)

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DISCUSSION

SACHS There is one small point which I did not quite follow in Mr Hamerton's really fantastically beautiful slides. In the slide of the pachytene stage (Fig. 2) the autosomes were all very fuzzy whereas the sex chromosomes were not fuzzy at all, but at diakinesis (Figs. 3 and 4) they were all uniformly fuzzy Also in addition to these regions which you call pairing regions which are obviously lying next to each other there was in the slide a large *heteropycnotic lump on top Is this also part of the sex bivalent* or is it part of an autosome?

HAMERTON It has been observed in other animals that at pachytene the sex chromosomes are frequently precocious and are condensed before the autosomes. This precocious condensation of the sex chromosomes is well known in other mammals—Slizynski has observed it in the mouse The heterochromatic region referred to by Dr Sachs is definitely part of the sex chromosome, and it can be observed in the best preparations to have a double structure.

SACHS My reason for raising this point, not that I of course doubt the observation, is that I find it very difficult to see how one can really determine whether the pairing and chiasma formation of the sex chromosomes is of the nature one knows to exist between the autosomes because you can never get all the chromosomes at exactly the same stage and giving the same picture. Also that in mouse and in man the sex chromosome *constitution is not the same as you never have a piece outside the* vesicle and a piece inside the vesicle in the latter

HAMERTON This again is debatable. Slizynski has worked extensively on the meiotic stages in the mouse and has claimed that in fact the sex chromosomes are partly in the vesicle which is

the same cellular structure (Leuchtenberger *et al* 1956a) as will be presented in this report. While there is no time to discuss any of the techniques employed I would like to show briefly the micro-

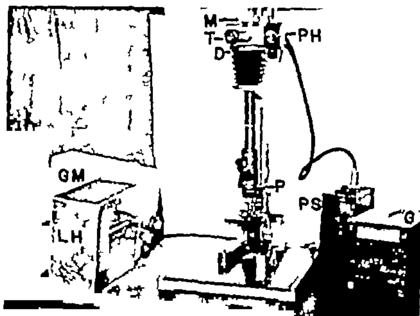


Fig. Visible microspectrophotometer (modified after Pollister and Moses)

D	Diaphragm Lever	P	Prism
G	Galvanometer	PH	Photocube Housing
GM	Grating Monochromator	PS	Power Supply
LH	Lamp Housing	T	Telescope
M	Mirror Rotation Knob	VS	Voltage Stabilizer

spectrophotometer (Fig 1) which, as you see, is just a microscope combined with a photometric device which allows absorption measurements of light in a single cell structure. The amount of light absorbed at a wavelength specific for the chemical components to be investigated permits the quantitation of the substance. For the determination of the dry weight a Baker interference microscope was utilized which is shown in the next figure (Fig 2) Here the measurement of the retardation of light introduced by the cell structure permits the determination of the dry mass of *a single cell structure such as a sperm nucleus. Usually* the phase shift of retardation of light is obtained by matching visually the density of the background and structure but as you can see, we combined the microscope with a photometric device so that objective matching of densities is possible

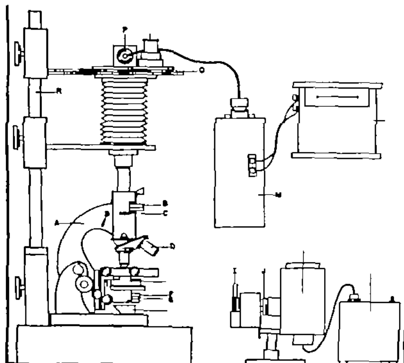


Fig. 2. Schematic diagram of Baker interference microscope with photometric attachments.

A	Baker interference microscope	K	Lamp housing with AH_4 mercury vapour bulb
	Rotating polarimeter analyser	L	Transformer
C	Quarter-wave retardation plate	M	Phototube power supply
D	Shearing system objective	N	Galvanometer
	Condenser	O	– Upper diaphragm
F	– Condenser diaphragm	P	– Phototube housing
G	Polarizing plate	Q	– Telescope
H	– Prism	R	– Supporting stand
	Wratten filters Nos. 58 and 77A		
J	Lamp diaphragm		

II RESULTS

For a considerable number of years we have applied these techniques to the study of the desoxynucleoproteins in cells of normal human tissues, and in a variety of pathological conditions such as tumours, virus diseases hormonal disturbances (for review see Leuchtenberger 1957 1958 Leuchtenberger and Leuchtenberger 1957) One problem which we have studied extensively for the last seven years is that of male sterility Here we found that

the same cellular structure (Leuchtenberger *et al* 1956a) as will be presented in this report. While there is no time to discuss any of the techniques employed, I would like to show briefly the micro-

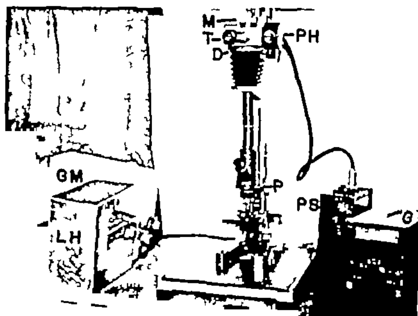


FIG. Visible microspectrophotometer (modified after Pollister and Moses)

D – Diaphragm Lever
G Galvanometer
GM – Grating Monochromator
LH Lamp Housing
M Mirror Rotation Knob
P Prism
PH Phototube Housing
PS – Power Supply
T Telescope
VS – Voltage Stabilizer

spectrophotometer (Fig 1) which as you see, is just a microscope *combined with a photometric device which allows absorption* measurements of light in a single cell structure The amount of light absorbed at a wavelength specific for the chemical com ponents to be investigated permits the quantitation of the sub- stance For the determination of the dry weight a Baker inter ference microscope *was utilized which is shown in the next* figure (Fig 2) Here the measurement of the retardation of light introduced by the cell structure permits the determination of the dry mass of a single cell structure such as a sperm nucleus. Usually the phase shift of retardation of light is obtained by matching visually the density of the background and structure but, as you can see, we combined the microscope with a photometric device so that objective matching of densities is possible.

MEAN AMOUNTS OF DNA (FEULGEN MICROSPECTROPHOTOMETRY) IN 3000 SPERMATOZOA OF 55 FERTILE BULLS

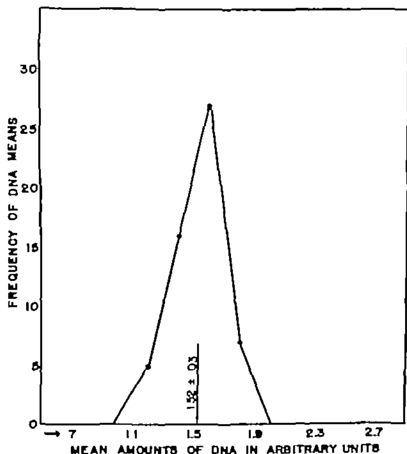
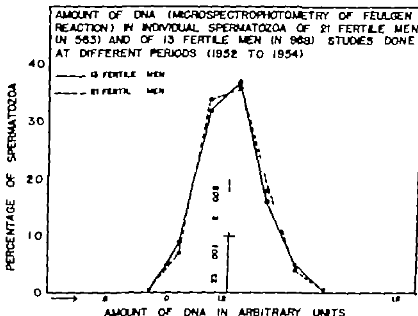


FIG. 4.

linked to a basic protein which has a conspicuously high amount of arginine in bulls, the question arose whether the proteins in human sperm nuclei would show a similar pattern. For these studies several thousand individual sperm nuclei of 34 fertile men

at least one type of male sterility in man and cattle can be explained on the basis of deficient amounts of DNA in spermatogenic cells and mature spermatozoa. Fertile men as well as fertile bulls have a constant haploid amount of DNA while sterile men and bulls have a deficient amount of DNA and show a great variability in their DNA content (Leuchtenberger *et al* 1953 1955, 1956a, b c, d Leuchtenberger and Schrader 1955)



N NUMBER OF SPERMATOZOA MEASURED

MEAN AMOUNTS OF DNA REPRESENTED BY VERTICAL LINE

FIG. 5.

Although this report is not concerned with the problem of fertility nevertheless I would like to show briefly two figures which demonstrate (Figs. 3 and 4) the constancy of the DNA content in spermatozoa for both fertile men and fertile bulls because it was their DNA constancy which led us to investigate the behaviour of the proteins in the sperm nuclei I think these figures not only show the striking constancy of DNA from sperm to sperm in fertile mammals but demonstrate also the similarity in the quantity of DNA between humans and bulls Since we know that sperm nuclei are made up predominantly of DNA

DETERMINATION OF AREA, DRY MASS (INTERFERENCE MICROSCOPY) DNA AND ARGININE (FEULGEN FAST GREEN MICROSPECTROPHOTOMETRY) IN THE SAME HUMAN SPERM NUCLEI

SPERM NO	μ^2	10^{-9} MGM			DNA/ARGININE RATIO
	AREA	DRY MASS	DNA	ARGININE	
1	6 6	6 7	2 36	1 25	1 89
2	8 0	6 2	2 42	1 32	1 83
3	8 6	6 6	2 36	1 22	1 93
4	8 0	6 2	2 48	94	2 64
5	7 6	7 1	2 68	1 11	2 41
6	7 6	6 8	2 40	1 24	1 94
7	7 1	6 4	2 48	1 24	2 00
8	7 1	6 0	2 60	1 18	2 20
9	7 1	6 0	2 36	1 06	2 25
10	7 6	6 1	2 42	1 01	2 40
11	8 6	6 3	2 42	1 01	2 40
12	8 0	6 8	2 36	1 15	2 05
13	7 6	5 3	2 74	1 17	2 34
14	8 0	6 5	2 56	91	2 81
15	8 0	6 5	2 29	1 21	1 88
16	6 2	6 2	2 40	1 17	2 05
17	7 1	6 2	2 18	1 01	2 16
18	8 0	6 5	2 74	1 17	2 34
19	7 6	6 1	2 36	1 25	1 89
20	9 1	6 7	2 64	1 14	2 32
MEANS	7 7	6 35	2 46	1 14	2 15
S E	± 18	± 09	± 03	± 03	± 5

FIG. 6.

nuclei It can be seen that there is relatively little [illegible] sperm to sperm in dry mass DNA, and arginine. [illegible] further note that the ratio between DNA and arginine [illegible] very little from the mean ratio of approximately [illegible] now the table above (Fig 6) which gives a chara[illegible] of these values for human sperm nuclei a similar [illegible]

behaviour from sperm nuclei to sperm nuclei can be seen. However there is one very obvious difference between the bull sperm and human sperm nuclei that is their content of arginine which is significantly higher in bull sperm than in the human sperm nuclei. In the table below (Fig 7) a comparison of the mean values computed on the basis of an analysis of over 22 000 individual sperm nuclei is shown. It is evident that there is no significant difference in the dry mass and only a small difference in the

DNA, ARGININE AND DRY MASS VALUES FOR BULL AND HUMAN SPERM NUCLEI *

METHOD	TYPE OF MAMMAL	10^{-9} MGM			
		DNA	ARGININE	DNA/ARGININE	DRY MASS
MICROSPECTROPHOTOMETRY (FEULGEN, FAST GREEN) AND INTERFERENCE MICROSCOPY	BULL	3.04*	2.07*	1.47	7.1
	HUMAN	2.50*	1.20*	2.08	6.6
BIOCHEMISTRY (VENDRELY AND VENDRELY)	BULL	3.20	2.16	1.48	—
	HUMAN	—	—	—	—

* ANALYSIS DONE ON OVER 22,000 INDIVIDUAL NUCLEI

FIG. 7

DNA content between bull sperm and human sperm nuclei. However the arginine content of the bull sperm nuclei is nearly 80 per cent higher than that of the human sperm nuclei leading to significant differences in the DNA/arginine ratios. A comparison of a biochemical analysis done on the same material by the Vendrelys (1953) shows indeed an amazingly good agreement between their data done on bull sperm suspensions and our data done on individual bull sperms, and certainly lends confidence to the human data. This relationship between DNA arginine and dry mass as established by microspectrophotometry and interference microscopy permits an additional analysis of which a characteristic example is given in Fig 8 Since the dry mass of sperm nuclei can be accounted for by DNA and proteins, the proteins can be easily computed by subtracting the DNA from the dry mass. In the bull sperm we have 7×10^{-9} mg dry mass,

3×10^{-9} mg DNA, therefore 4×10^{-9} mg protein or approximately a ratio of 40 to 60 per cent. In the human sperm we have 6·6 dry mass, 2·5 DNA, therefore 4 protein and again approximately 40 per cent DNA, 60 per cent protein as in the bull

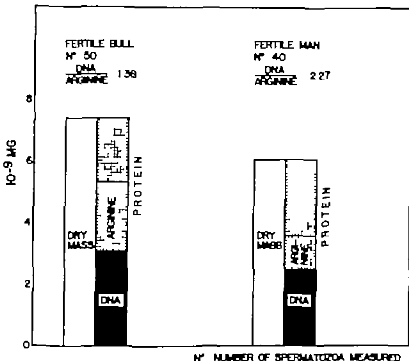


FIG. 8.

sperms. However the arginine content of the proteins in bulls is approximately 50 per cent while in humans it is only 30 per cent, and it is therefore the *quality* of the proteins which is strikingly different in human as compared with bull sperm nuclei. Although the implications of this finding *cannot be assessed at the present* time, nevertheless the difference in the composition of the proteins between human and bull sperm nuclei is of interest if we take into consideration that the desoxynucleoproteins in sperm nuclei themselves are the genetic material or at least the carrier of the genetic material. The impossibility of cross fertilization between

behaviour from sperm nuclei to sperm nuclei can be seen However there is one very obvious difference between the bull sperm and human sperm nuclei, that is their content of arginine, which is significantly higher in bull sperm than in the human sperm nuclei. In the table below (Fig 7) a comparison of the mean values computed on the basis of an analysis of over 22,000 individual sperm nuclei is shown. It is evident that there is no significant difference in the dry mass and only a small difference in the

DNA, ARGININE AND DRY MASS VALUES FOR BULL AND HUMAN SPERM NUCLEI[a]

METHOD	TYPE OF MAMMAL	10^{-8} MGM			
		DNA	ARGININE	DNA/ARGININE	DRY MASS
MICROSPECTROPHOTOMETRY (FEULGEN, FAST GREEN) AND INTERFERENCE MICROSCOPY	BULL	3.04	2.07	1.47	7.1
	HUMAN	2.50	1.20	2.08	6.6
BIOCHEMISTRY (VENDRELY AND VENDRELY)	BULL	3.20	2.16	1.48	—
	HUMAN	—	—	—	—

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of individual chromosomes. Have you got any further in the investigation of this particular point?

LEUCHTENBERGER Yes, we have done some investigations on that, not in humans but in cattle, and have found that the chromosomal numbers are still normal and it is the DNA which may vary so that the chromosomes may not all contain the same amounts of DNA. Deficient amounts of DNA cannot be explained on deficient chromosomal number

6

THE GENETIC IMPLICATIONS OF NUCLEAR SEXING

LEO SACHS Ph.D and MATHILDE DANON Ph.D
Department of Experimental Biology
Weizmann Institute of Science,
Rehovoth, Israel

In the study of any organism, the geneticist is interested in finding out where genetic factors are involved and how they produce their effect. In the field of human sexual development, he is therefore interested in finding evidence of chromosomal abnormalities and gene mutations and in studying the relationship

FREQUENCY OF "INTERSEX" CONDITIONS
AND PROBABLE (+) AND CERTAIN (+ +) GENETIC FACTORS

CONDITION		% OF CASES (from Money and Wilkins et al)	EVIDENCE FROM PEDIGREES
True hermaphrodites		10	–
Male Pseudohermaphrodites	simulating males	27	+
	simulating females	14	+ +
Female Pseudohermaphrodites	with adrenal hyperplasia	46	+ +
	without adrenal hyperplasia	3	–
Sex reversal in Klinefelter syndrome			+
Sex reversal in agenesis of the gonads			+

Fig.

between their occurrence and developmental abnormalities since this can help to elucidate not only the development of the abnormal but also of the normal organism

The study of pedigrees was until recently the only source of evidence of possible genetic factors in human intersexuality This has indeed been a fruitful approach, and famillial Incidence has been reported in many types of intersexes (Fig 1) a definite mode of inheritance being known in two intersexuallty syndromes

the adrenogenital (Bentinck *et al* 1952) and the testicular feminization" syndrome (Taillard and Prader 1957)

The method of nuclear sexing and its application to human intersexes for the introduction of which we are indebted to Professor Barr and his colleagues (Barr 1954) has disclosed a number of facts, which stress to an even greater extent the importance of genetic factors (Danon and Sachs, 1957) Of particular interest because of their novelty are

(*a*) The existence of mosaic organisms having patches of cells of variable extent presenting either the female or the male chromatin pattern (Danon and Sachs 1957) Such somatic mosaics could originate either through the fertilization of an X ovule (presumably binucleated) by two sperms (with an X and a Y) or through the loss at a later stage of development of an X chromosome in a line of somatic cells. Such organisms are not gynandromorphic, since in gonadal agenesis there is no male hormone to bring out the male phenotype It can be assumed that real gynandromorphs could exist in humans only among true hermaphrodites.

(*b*) The cases with excessive numbers of somatic nuclei with 3 chromocentres (as compared to normal males and females) (Danon, 1957) They belong to a clearly inherited syndrome the "testicular feminization" syndrome, which constitutes a well separated clinical entity (Hamblen *et al.*, 1951 Schneider *et al.*, 1952 *Morris 1953*) and are characterized by a resistance to androgens (Wilkins, 1950 Prader 1957)

(*c*) The cases with complete sex reversal from a female sex chromosome constitution to a male phenotype, belonging clinically to the true" Klinefelter s syndrome (Heller and Nelson 1945) The distinction between "true and "false Klinefelter's syndrome which can be made histologically has been found to correspond always to the XX and XY sex chromosome constitution respectively (Nelson 1956 Riis *et al.*, 1957 Ferguson-Smith *et al* 1957) The existence of familial occurrence among them supports the possible genetical basis of this anomaly (Reifenstein, 1948 Nadler *et al* 1950 Grumbach *et al.*, 1957 Pasqualini *et al.*, 1957 Stewart *et al.*, this symposium) which reminds one of a genic induced sex reversal (Danon and Sachs, 1957) that is known to exist in Drosophila (Sturtevant, 1945)

The above facts can be utilized in order to determine when and how genetic factors act in human sexual development. When the type of development of the gonad is affected, they must act very early since the testis is believed to start secreting androgens in humans at the seventh week of intra-uterine life. We have

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	without adrenal hyperplasia	3	—
Sex reversal in Klinefelter syndrome			+
Sex reversal at agenesis of the gonads			+

FIG.

between their occurrence and developmental abnormalities, since this can help to elucidate not only the development of the abnormal but also of the normal organism.

The study of pedigrees was until recently the only source of evidence of possible genetic factors in human intersexuality This has indeed been a fruitful approach and familial incidence has been reported in many types of intersexes (Fig 1) a definite mode of inheritance being known in two intersexuality syndromes

chromosome or chromosome segments The mosaics are extremely interesting from this point of view because this is the sort of evidence which led one to assume that you might have a mixed cell composition.

RIIS Did you find the mosaic pattern in mesenchymal tissue, in connective tissue, and in blood forming organs or was it only found in epithelial tissue?

SACHS That would be an extremely interesting thing to find out, but unfortunately the only materials available were biopsies of skin. If one could obtain tissues from different parts of the body of these patients one might be able to answer that question. It is an extremely important point, but unfortunately we have no particular evidence at the moment.

FORD I would like to suggest that if anyone does have the opportunity of examining a case which might be suspected to be a mosaic, examination of the hæmopoietic tissues might be misleading There is evidence of competitive replacement of one cell genotype by another in mice in the hæmopoietic system so that if you did have a mosaic produced (XX, XO) one type might very well outgrow the other completely and therefore the hæmopoietic tissues would not give you definite evidence. I should think that epithelial tissue would be much more fixed and very much safer

SEX CHROMATIN IN LYMPHOCYTIC CELLS FROM PERIPHERAL BLOOD

POVL RIIS M.D
Medical Department N, Copenhagen County Hospital,
Hellerup, Denmark

THE sex-chromatin in intermitotic somatic nuclei can be detected in leptochromatic nuclei only When more or less pachychromatic nuclei form part of a given cellular material they are often omitted in diagnostic counting Certain types of cells are pachychromatic to such a degree that sex detection is impossible. The small lymphocytes from peripheral blood are examples of such cells

The questions leading to the present work were whether a sex chromatin body could be unmasked in these cells, and if such sexing was possible, could an impression of the way an eventual unmasking took place be obtained

Various phases of cellular function are known to be related to *the intranuclear protein metabolism The latter is further known* to manifest itself in certain morphological changes in the chromatin pattern. Consequently a profound change in cellular growth and function was primarily induced in small lymphocytes from peripheral blood with the secondary aim of unmasking a sex specific part of the chromatin

The technique employed was a 24 hour incubation of human *buffy coat fragments in a coagulum of homologous serum* (Riis, 1957a, b) By carefully watching the various stages of lymphocytic emigration a gradual and regular increase in nuclear size and outline was observed. Synchronously with this development a regular splitting of the coarse chromatin blocks into minor fragments could be detected. The end result in cells from male individuals was a rather leptochromatic nucleus with multiple small (less than 1 μ) peripherally located chromatin condensations or a complete disappearance of particulate chromatin as a whole. In cells from female individuals a similar dissolution took place *with the exception that sex-chromatin was eventually un*masked" The size, form, and location of this body was just like that of the sex-specific chromatin in epithelial cells. A small vacuole was seen in preparations of optimal quality (Figs. 1 and 2)

The significance of the present work is not of course to introduce a new method of cellular sex determination for routine clinical work. For this purpose the methods based on examination of oral or urogenital desquamated epithelial cells are much more

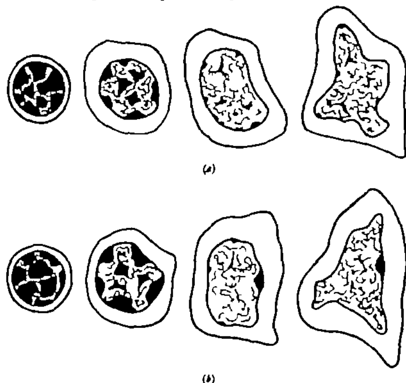


Fig. (and *b*) Drawings of nuclear changes in male lymphocyte (top) and female lymphocyte (bottom) during incubation.

convenient. It adds however a little theoretical knowledge of the relation between sex specific and unspecific chromatin of mesenchymal cells Furthermore the application of similar principles on the sex-chromatin studies in hitherto unsexable cells from certain animal species might be valuable.

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( )

20μ



FIG. (and b) Photomicrographs and drawings of male lymphocytes (top) and female lymphocyte (bottom) after incubation. The arrow indicates the unmarked sex chromatin.

DISCUSSION

ROBERTSON SMITH I would like to ask Dr Riis what proportion of the lymphocytes after incubation show this differentiation?

RIIS In good preparations, almost every lymphocyte changes its morphology and shows this changing arrangement of nuclear chromatin, but only about 30 per cent show this distinct nuclear condensation in female cells after 24 hours. The frequency might be much higher if you continue the culture for several days.

LENNOX Why can t you sex monocytes? They appear to have favourable nuclei.

RIIS I have tried to sex monocytes in peripheral blood. There might be a sex difference but, using the strictest criteria, I would not rely on monocytes alone for sexing peripheral blood

KLINGER In my experience after acid hydrolysis we cannot sex monocytes.

SIEBENMANN May I ask Dr Riis if he has made the same observation of chromatin condensation in tissue sections also?

RIIS No it is rather difficult to work with this kind of cell in solid preparations as you only obtain the highest degree of differentiation when they are allowed to stretch along glass surfaces.

BARR Your comment that incidence of sex chromatin under these conditions is high brings us back in an important respect to Slizynski s paper You have been sexing whole nuclei and I think this is an advantage The tables of the incidence of sex chromatin given in various papers are rather misleading So far as our own tables are concerned we never intended to imply the accuracy that one might infer from the fact that there are actual figures down there Mechanical and technical factors are so very important in producing those results In amnionic membrane for example when staining the whole tissue one can see through the thickness of the entire nuclei and the incidence is practically 100 per cent as Mr Klinger has said. Another instance where the situation is favourable is the lateral geniculate body in the brain. The nerve cells there are very large and we had 12 μ sections and we probably had most of the nucleus in the section. We stained with thionin which because of its metachromatic properties is very good from this point of view as the sex chromatin stains a little different shade from the nucleolus and the Nissl material These two factors combined made it very favourable and the incidence there was of the order 96–98 per cent. I want to offer a word of caution about accepting the figures given as being a real reflexion of the situation in the actual nuclei I don t think they are in many instances.

(a)

20μ



Fig. 2. (and b) Photomicrographs and drawings of male lymphocytes (top) and female lymphocyte (bottom) after incubation. The arrow indicates the unmarked sex chromatin.

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C. LEUCHTENBERGER I wonder if a cytochemical technique would help you very much. Have you ever tried to use the enzyme desoxyribonuclease which digests differentially? In cytology we usually use it to show very nicely heterochromatin material. If you used it on the lymphocytes you could get rid of the other chromatin You might be able to show the sex chromatin in the monocytes also because after all it is only masked isn t it?

APPLICATION TO THE STUDY OF INTERSEX AND RELATED STATES

CHAIRMAN DR. PETER BISHOP

8

THE SEX CHROMOSOMES AND THE DEVELOPMENT OF HUMAN INTERSEXES

MATHILDE DANON Ph.D (read by L. SACHS)
Department of Experimental Biology
Weizmann Institute of Science
Rehovoth, Israel

SINCE 'nuclear sexing' methods were applied to a wide range of abnormal types of sexual development, it has become possible to classify these on the basis of their sex chromosome constitution versus type of gonads and phenotype (Fig 1)

The intersexes with an XX sex chromosome constitution show a range of intermediate phenotypes between male and female, including complete sex reversal with reversal of the type of the gonad (Klinefelter's syndrome) (Heller and Nelson 1945) The intersexes with an XY sex chromosome constitution also show all degrees of phenotypic sex reversal, the complete reversal being probably rare since such a case is to be presented for the first time by D J B. Ashley at this Symposium. In cases of gonadal agenesis, whatever the chromosome constitution, the female phenotype develops thus being in agreement with theories evolved from Jost's experiments on embryonic castration (Jost, 1950) In one type of male pseudohermaphroditism (testicular feminization syndrome) (Hamblen *et al* 1951 Schneider *et al* 1952 Morris, 1953) for which cytological evidence indicates an abnormal sex chromosome constitution of the type XXY (Danon, 1957) a peculiar development occurs which includes active testes with a characteristic female body differentiation.

We will mainly consider here those cases of intersexuality in which the gonads have been affected as shown by their having undergone a differentiation contrasting with the sex chromosome constitution or by being altogether absent i.e the reversals of sex as in Klinefelter's syndrome gonadal agenesis, and the testicular feminization syndrome Their very early onset, their reported familial incidence, and the frequent association of two of these syndromes—agenesis and Klinefelter's—with other inherited anomalies (Caflish, 1952 Nadler *et al* 1950 Bamoe 1956 Grumbach *et al* 1957) have already led many authors to suggest

humans) Both cortical and medullary elements are determined —the medulla as a potential testis and the cortex as a potential ovary The problems raised by the differentiation of the gonad up to this stage of development are similar to those raised by

GENOTYPE, MORPHOGENESIS OF THE GONADS AND PHENOTYPE

XX/AA	XY/AA
NORMAL	
Genetically determined morphogenesis	Genetically determined morphogenesis
Foetal secretions (?)	Foetal secretions
Phenotype ♀	Phenotype ♂
KLINEFELTER SYNDROME	
Genetically determined morphogenesis	
Foetal secretions	
Phenotype ♂	

I the gonad the heavy arrows indicate development and the light arrows regression.

Fig.

other morphogenic processes in an embryo. Animal experiments based on delicate hormone assays (Padoa, 1947) transplantation (Humphrey 1928a, b 1933 Buyse, 1935 Willier 1939 Torrey 1950 Holyoke, 1956) and cultivation *in vitro* (Wolff and Haffen, 1952) have indeed all shown that the medullary primordium in males and the cortical primordium in females are very early determined with respect to their future differentiation, long before any morphological differences appear This led to the basic assumption that the sex chromosome constitution plays the determining role in deciding the differentiation of the gonad in normal development (Fig 2) The XX sex chromosome

constitution gives a leading role to the cortical primordium, while the XY constitution gives a leading role to the medulla and in both cases the heterologous primordium regresses All these *events represent the expression of intrinsic cellular potentialities* determined by genetic factors. The development of the medulla includes the capacity of the early secretion of the male sex hormone responsible for the differentiation of the primary and secondary sex characters leading to the male phenotype The normal female phenotype presumably develops autonomously without the need of hormones (Jost, 1950)

Also found experimentally in different classes of animals, is the important fact that when either the cortical primordium in females or the medullary primordium in males is inhibited or destroyed the other primordium develops and produces a gonad according to its own determination, thus producing a sex reversal (Ponse 1924 Witschi, 1929 Domm, 1939)

On the basis of these facts one can visualize the type of mechanism through which genetic factors could give rise to abnormal or reversed gonadal differentiation.

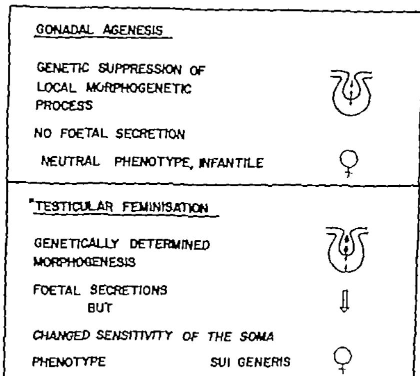
In the case of Klinefelter's syndrome the formation of the undifferentiated Anlage may be normal, but a genetic factor present in their cell nuclei changes the potentialities of either the cortical or the medullar primordium, weakening the capacity of the former to form an ovary or reinforcing the capacities of the latter *The result of either event would be the same the formation of a testis* (Segal and Nelson 1957) The known bipotentiality of the soma in its response to hormones explains that this XX organism which is since the very beginning subjected to hormones released by a testis, develops male primary and secondary sexual characteristics From the genetical point of view such a supposed genetically induced change in the direction of differentiation is not an unlikely event a number of simply and typically inherited genes are known in Drosophila (the so-called "homeotic genes) which are capable of bringing about much more striking changes in morphogenesis such as changing the differentiation of one segmental organ into another i.e. an antenna into a leg or a haltere into a wing (Villee, 1942 Herskowitz, 1949) They obviously act through releasing the expression of intrinsic properties Intrinsic potentialities of development are demonstrable in the recessive component of the gonad when its dominant component is eliminated experimentally (Ponse, 1924 Witschi, 1929 Domm, 1939) We would also recall the parallel which has already been mentioned (Danon and Sachs, 1957) between the human type of sex reversal found in

Klinefelter syndrome and a genetically conditioned sex reversal in Drosophila due to a single recessive autosomal mutant gene, which induces a complete reversal from female to male including gonads and germ cells, the testes remaining small and the flies sterile (Sturtevant, 194) Witschi, Nelson, and Segal (1957) have offered an alternative interpretation for the mechanism giving rise to a sex reversal of the type of Klinefelter syndrome. They suggest that an impairment in the number of germ cells could be the causative factor for the involution of the cortex and consequently for the reversal of sex. Although the causal importance of the lack of germ cells is difficult to ascertain in this case the experimental evidence available suggests the dependence of the germ cells upon the structural elements of the gonad and not vice versa their early destruction by irradiation produces normal but sterile ovaries and testes (Bonoure 1937 Dulbecco 1946)

In gonadal agenesis, a gen tical factor could act in suppressing the development of the whole gonadal "Anlage" (Fig 3) There are many cases known of gene-induced suppressions of organs including suppression of whole segments of limbs (ectromelia in mammals) Deletion of a chromosome or part of a chromosome —a possibility discussed elsewhere and suggested by the existence of mosaic organisms (Danon and Sachs, 1957)—could account for the frequently associated anomalies.

In the 'testicular feminization syndrome one deals with a remarkable condition in which a male gonad is functionally active in an entirely though somewhat atypical feminine soma. The genetical basis of this syndrome is clearly established by the existence of numerous pedigrees showing a definite mode of inheritance (Taillard and Prader 1957) Nuclear sexing has indicated that there is a particular sex chromosome constitution (*Danon, 1957*) *Here one can postulate that the peculiar chromosome* constitution is compatible with a medulla predominating during differentiation and that this results in the formation of a testis secreting the male hormone, but that the responsiveness of the somatic tissues to hormones is altered so that there is no masculinization The lack of response to hormones is in fact confirmed by the failure of these intersexes to respond to the administration of testosterone (Wilkins, 1950 Prader 1957) There is also evidence in animal genetics of genetical factors affecting the responsiveness of somatic tissues (Stern, 1955) and the different sensitivity of male and female tissues to a given dose of hormone in some experimental conditions, patently illustrates the genetical conditioning of somatic responsiveness (Greene and Burrill 1942 Burns, 1956)

In the present discussion we have used the basic premise that the difference in sex chromosome constitution between male and female determines the relative developmental potentialities of the *cortex and medulla of the gonadal primordium.* We have tried



In the gonad the heavy arrows indicate development and the light arrows regression.

FIG. 5.

to extend this concept to cases of human intersexuality in which genetical ætiological factors are involved with certainty or with some likelihood. We have pointed out that the genetic modes of action that *have to be supposed in those disorders are paralleled* by similar effects of genetic factors, whether on the sexual development or in other fields of morphogenesis in animals submitted to experimental analysis. We hope that we have been able to show that in the absence of experimental data in humans, such comparisons may be a fruitful approach towards the understanding of some puzzling cases of abnormal human development.

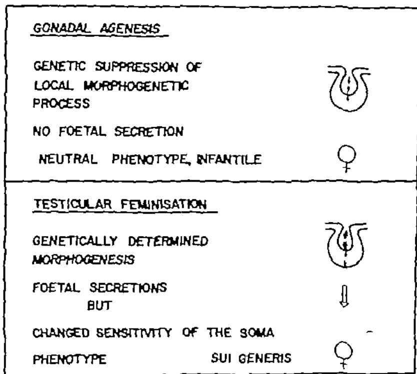
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DISCUSSION

This paper was discussed after papers 9 and 10 See page 73.

In the present discussion we have used the basic premise that *the difference in sex chromosome constitution between male and* female determines the relative developmental potentialities of the cortex and medulla of the gonadal primordium. We have tried



In the gonad the heavy arrows indicate development and the light arrows regression.

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judged by negative Ashbel-Seligman reaction. Additional characteristics, regarded as secondary are the occurrence of patchy calcification of lipid crystals, and of multinucleated giant cells in the hyperplastic cortex.

The cortical hyperplasia and the accumulation of lipid material reminds one of the adrenal changes observed in rats treated with Amphenone As this substance is known to interfere with the synthesis of adrenal steroids, the morphological appearance suggests a primary metabolic disorder of the adrenal cortex in our syndrome.

This is supported by our studies on the adrenal function, summarized in Table II The results leave no doubt as to the existence of a general adrenal insufficiency The adrenal cortical hyperplasia shows clearly that this insufficiency is not caused by a primary pituitary disorder but represents a primary adrenal disorder Additional evidence for a primary disorder of the adrenal cortex is the complete lack of response to exogenous ACTH.

Such a primary adrenal insufficiency can only be explained by a metabolic defect in the adrenal steroid synthesis (Table III) In the adrenogenital syndrome the enzymatic block is in one of the last steps of this synthesis namely between 17 OH progesterone and cortisol. This leads to the accumulation of intermediate steroids and their metabolites but not to the accumulation of lipids and cholesterol In contrast to this, adrenals with lipid hyperplasia are characterized by an enormous accumulation of lipids and cholesterol and by a striking lack of ketosteroids as judged from functional and histochemical studies. This suggests an enzymatic block in one of the first steps of steroid synthesis. In other words, there are reasons to place the primary metabolic defect of lipid adrenal hyperplasia in the first stages and the primary metabolic defect of the adrenogenital syndrome in the last stages of adrenal steroid synthesis.

This brings us to the last problem, how to account for the female genitalia in boys. How can this defect of genital development be related to the defect of adrenal steroid synthesis? The most reasonable explanation is to postulate that the enzymatic defect of steroid synthesis affects not only the adrenal steroids but also the testicular steroids The inability of the foetal testes to produce testicular steroids readily explains the lack of male genital development The synthesis of testosterone is very similar to the synthesis of the adrenal steroids. Both start from acetate and cholesterol and involve the transformation of delta-5-steroids into delta-4 ketosteroids (Table III) An enzymatic defect in the

TABLE I

CONGENITAL ADRENAL INSUFFICIENCY WITH LIPID ADRENAL HYPERPLASIA

Patients		2*	3	4	5	6
Gonads	♂	♂	♂	♀	♀	♀
Chromosomal sex *Genitalia*	♀	♀	♂ ♀	♀	♀	♀ ♀
Age at death (months)	½		½	2	3	8
Clinical symptoms						
Failure to thrive	+	+	+	+	+	+
Apathy	+	+	+	+	+	+
Anorexia	+					+
Vomiting	+	+	+			+
Diarrhoea	+		+	+		−
Weight loss	+		+	+	+	+
Pigmentation			−		+	+
Weight of the adrenals (gm)	†		7 5	5	6	5-6†

References of patients to 6 † *Treated with cortisone*

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TABLE II

ADRENAL FUNCTION IN TWO INFANTS WITH CONGENITAL LIPID HYPERPLASIA OF THE ADRENALS

The clinical symptoms (Table I) and the success of substitution therapy are suggestive of adrenal insufficiency

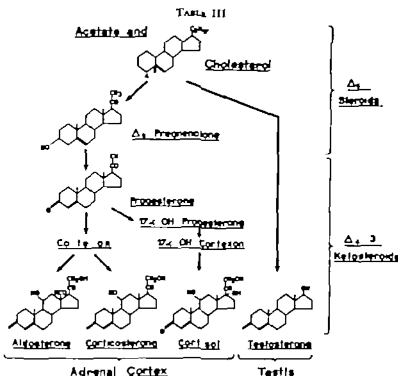
Androgens	No clinical androgenic symptoms. 17-KS low no response to ACTH. Biological assay of adrenal tissue negative.
Œstrogens	No clinical oestrogenic symptoms. Vaginal smear immature Biological assay of adrenal tissue negative.
Glucocorticoids	17-OH-C low no response to ACTH. Eosinophils normal to high, no response to ACTH. Blood sugar in the normal range. Pigmentation, disappearing with cortisone.
Electrolyte regulation	Serum-K increased with typical E.C.G. Serum-Na decreased. Both corrected by NaCl and steroids.

early steps of steroid synthesis might therefore well affect both pathways in the same manner

In summarizing we believe that "congenital lipid hyperplasia of the adrenals" is caused by a hereditary enzymatic defect in the early steps of steroid synthesis in the adrenals and in the testes, leading to adrenal insufficiency and preventing the normal development of male genitalia.

A detailed report about all six cases with a complete discussion is in preparation.

TABLE III



Normal pathway of steroid synthesis in the adrenal cortex and in the testis. In the congenital adrenogenital syndrome there is late block preventing the transformation of 17α-OH-progesterone into cortisol. In lipid hyperplasia there is an early block, preventing possibly the transformation of cholesterol to pregnenolone or the transformation of delta-5-steroids into delta-4-ketosteroids.

DISCUSSION

This paper was discussed with papers 8 and 10 See page 73

PROBLEMS IN INTERSEXUALITY CONCERNING THE SEXUAL DUCTS IN TRUE AGONADISM, GONADAL DYSGENESIS AND TURNER S SYNDROME

C. OVERZIER, Prof. Dr med.
Oberarzt der Medizinischen Universitätsklinik,
Mainz, Germany

DURING the past few years we have seen many individual observations of gonadal duct disturbances in which the sex has been determined from the nuclear chromatin nodules (Barr and Bertram 1949 Barr *et al* 1950 Carpentier *et al* 1955 Graham and Barr 1952 Lupatkin and Prader 1956 Marberger and Nelson, 1954 Moore and Barr 1955 Prince 1952 Prince *et al* 1955) or by leucocytic examination (Davidson and Smith 1954 Kosenow and Schönenberg 1956 Kosenow and Scupin, 1956 Sohval and Soffer 1952 Wiedemann *et al* 1956 Wiedemann *et al* 1955) We feel it now essential to depart from older systems (Kermauner 1924 Klebs-Sauerbeck, 1870) and to substitute a new one

As clinical observations (Overzier 1955c 1956c 1956d 1957) and animal experiment (Dantschakoff 1941 Jost, 1956 1957 Raynaud and Frilley 1947 Overzier 1956a) indicate the observed anomalous development of the sex ducts are due to developmental disturbance of the gonads and the manner of formation of the ducts depends on the time of this disturbance (Overzier 1956a) The clinical cases which we see often months or years after full development, vary because of the presence or absence of the hormonal factor and this variation may be quite extensive. We can and must trace this back to the manner of formation thus we must *look at this problem functionally*

We must distinguish between developmental disturbances of the gonads and those of the sexual ducts. Both can be disturbed in development either alone or together Stange (1956) some months ago performed a noteworthy experiment in an effort to produce gonadal deformities. *With regard to the sex ducts*, I believe that animal experiments (Dantschakoff 1941 Jost 1957

1956 Overzier 1956c Raynaud and Frilley 1947) and the observations on true hermaphrodites, with male sex ducts developed under the direction of the testis and female under that of the ovary (Overzier 1955c 1956c) have demonstrated that their development depends on a stimulation from the gonads the development of a normal sex duct presupposes normal gonads and not the reverse The vast majority of genital deformities are caused by faulty gonadal formation Isolated faulty duct formation on the other hand, is quite rare In other cases—in genetically female persons—a virilization can be seen due to hormonal stimulation from the adrenal cortex (Broster *et al* 1953 Overzier 1955a 1956d 1957 Philipp *et al.*, 1953 Prader 1954 Prader and Gurtner 1955) and in rare cases to the application of hormones to the mother in pregnancy (Overzier *et al* 1955) or to hormone producing tumours in the pregnant mother (Brentnall *1945a 1945b* Javert and Finn, *1951*)

In considering the possible determination of sex genetically a classification such as has already been outlined is necessary A hermaphrodite has a discrepancy between the sex of the gonad and that of the duct a true hermaphrod te when gonads of both sexes are confirmed histologically and a pseudohermaphrodite when one may equate the gonads to one sex and the relevant ducts to the opposite sex. As long as the histological formation of the gonads is well defined, we may with advantage retain the subdivisions for the moment. Above all *it can be shown that male* pseudohermaphrodites are genetically of the male sex and female pseudohermaphrodites of the female sex (Overzier 1957)

The significance of the nuclear examinations may best be demonstrated on the basis of the new findings in the so-called Klinefelter's syndrome histological gonadal formations apparently alike but on close inspection differing occur in the two types indicating the likelihood of a different manner of development (Jackson *et al* 1956 Nelson 1956 Overzier 1957 Plunkett and Barr 1956 Siebenmann, 1957) Research which Nelson (1956) has performed enables us to define our boundaries clearly in this respect.

A large number of cases requiring still further subdivision are those described as ovarian agenesis (Cutler and Silver 1953 Wilkins and Fleischmann, 1944) ovarian aplasia (Erskine and Rannie 1946 Petsche and Radlinger 1954) gonadal agenesis (Nelson, 1956) or recently gonadal dysgenesis (Gordon *et al.*, 1955 Grumbach *et al.*, 1955 Lemaire *et al.* 1955) Since one is referring to more than merely words it seems advisable to seek a clear and meaningful conception. One cannot challenge the

term gonadal dysgenesis as it expresses a disturbance in the development of the gonads The gonads are damaged at some period of the embryonic life, and are more or less destroyed. The term gonadal agenesis frequently used to describe this condition is unjustified and ovarian agenesis is even less warranted as only a small proportion of the cases are chromosomal females (Barr 1955 1956 Carpentier *et al* 1956 DéCourt *et al*., 1954 Ehrengut, 1956 1955a 1955b 1954 Grumbach *et al*., 1955 Hauser *et al* 1956 Kosenow and Schönenberg 1956 Lamy *et al*., 1957 Lenz 1956 1957 Neimann *et al*., 1956 Nelson, 1956 Overzier 1957 Polani *et al*. 1954 Schonenberg *et al* 1957 Sun and Rackoff 1956) * An agenesis in these cases really does not occur for in actual fact the gonads start to develop and later degenerate The Greek word "genesis (origin) is inapplic able here

In most cases only gonadal remnants, such as stroma, are found. One can, however deduce from development of the ducts the fact that the gonads were present at an earlier stage, because *the gonads stimulate the development of the sex ducts. This* capacity of the gonads to stimulate the development of the sexual system is the deciding factor (Overzier 1955c 1956a 1956c 1957) it may be added that one can find a deranged development of the sexual ducts in individuals normal in all other respects (Hauser *et al* 1956 Hoffenberg and Jackson 1957 Klinefelter *et al* 1942 Morris, 1953 Polani *et al* 1954 Schneider and McCullach, 1943 Schneider *et al* 1952 Swyer 1955 Greenblatt *et al* 1956 Gordan *et al* 1955) and in individuals with multiple deformities, e.g Turner's syndrome. Cases with Turner's syndrome have been frequently classified upon external deformities. In Turner's syndrome the gonads are affected only as part of the other disturbances, time and extent playing a significant role in the development of the duct system. From this point of view I cannot accept the recent gene-theory of Hoffenberg and Jackson (1957) postulating three independent genes for infantilism" 'shortness of stature and 'musculo-skeletal anomalies, etc." although I too am quite familiar with cases with only one or two of these defects. As a matter of interest the normal looking female" (with gonadal dysgenesis) can be seen to have cubitus valgus in the photograph these authors published "Normal looking" Turner's syndrome with shortness of stature, infantilism but no other anomalies has been described by Grumbach *et al* (1955) Ehrengut and Baitsch (1957) On the other hand there may be stigmata of Turner's syndrome, *such as webbing of the neck and*

* Total 367 cases 3 1 ♂ 75 ♀ (1 ⚥?)

cubitus valgus in women with a regular menstrual rhythm (Perloff and Stein, 1953 Rosin and Caflisch, 1951) From the clinical point of view it may be necessary to separate the different types but we should not postulate genetically different syndromes only because of absence or presence of one or other anomaly Findings like those of Prader (1957a) and Taillard and Prader (1957) showing testicular feminization in ten family trees indicate a hereditary influence in the development and allow us to postulate a special genetically determined syndrome. This concerns only the hereditary factors and not the functional causal origin of the phenotype.

The experimental results of early embryonic castration (Erskine and Rannie 1946 Jost, 1956 1957) in both sexes, leading to the formation of a female duct system, are well known This explains the reason why these cases with dysgenesis of the gonads —whether ovarian or testicular—are of female phenotype as long as the damage occurred early enough. Should damage occur to the testicular anlage at a later time, when a male duct system has already developed we are presented with cases with the multiple deformities of the Turner's syndrome but with a penis and testicular rudiments (Caflisch, 1952 Dorff *et al.*, 1948 Flavell, 1943 Greenblatt and Nieburgs, 1948 Halonen *et al.*, 1956 Jackson and Hoffenberg 1957 James, 1952 Mackenzie 1953 Martin 1947 McCullagh, 1948 Prunty *et al.*, 1953 Reforzo-Membrives *et al* 1949 Rossi and Caflisch, 1951 Solis and Schwartz, 1951 Sougin-Mibashan and Jackson 1953) These extragenital deformities may be absent cases of early testicular atrophy due to tubular fibrosis (Nelson, 1956) genetically male false Klinefelter's syndrome" or finally from earlier damage so-called functional prepuberal castrates' (Heller *et al.*, 1943) I would like, however to apply to the latter title a further subdivision to separate those cases in which testicular damage occurred in the embryo from those in which it occurred at a later period of development. Nelson (1956) has recently indicated, too that most of the cases which he observed occurred in an embryonic stage of development.

These cases are certainly relatively rare and unfortunately not too well known. Becker (1957) recently claimed as unique a case of agonadism a term which he has borrowed from me and *applied incorrectly* I believe that this case may be considered as a functional prepuberal castrate, with some deformities as in the Turner's syndrome. The occurrence of "*functional prepuberal castration*" during embryonic life may only be considered genetically amongst the many other possible disturbances.

Becker (1958) now accepts my opinion.

True agonadism which I have discussed (Overzier and Linden, 1956) is truly rare but of considerable importance in principle I have described two cases—sibs of 12 and 14 years—and later Philipp (1956) has described another case, confirming my findings and following my explanations. Another case to date unpublished, has been seen in Switzerland, while Carpentier (1956) who claimed to have two cases of his own was unfortunately incorrect in his assumption (1957) All three cases—my two and that of Philipp—were genetically male, but certainly one day a genetically female case of agonadism will be observed. These cases of true agonadism (here anorchism) are without any extragenital deformity Since the gonads have never started developing the Müllerian and Wolffian ducts are not stimulated and remain one beside the other at an early stage of development, thus a well developed epoophoron and a foetal tube are found These cases do not have a uterus, vagina, penis, or scrotum but only a very small penis-like structure with the orificium urethræ at its base

These apparently very different deformities can be explained and co-ordinated from a functional standpoint. A static system which differentiates individual types from one another as intrinsically different does not truly represent the state of affairs. I have summarized (Overzier 1956a) these thoughts in the *Theory of Initial and Permanent Induction of the Gonads* which states

"The first anlage of the gonads stimulates the first anlage of the Wolffian and Mullerian ducts (Initial Induction) whereas later through permanent induction from developed gonads the male or female duct system develops If a permanent induction is found lacking then, due to an Initial Induction stimulus, the development of the ducts of genetically female as well as genetically male individuals follows a female pattern When an Initial Induction is not present then the Müllerian and Wolffian ducts remain one beside the other undeveloped" (Overzier 1956a)

SUMMARY

The author attempts to develop a new systematic subdivision of a variety of intersexual types based on clinical observations, nuclear sex investigation, and animal experiments The gonads stimulate the development of the sex ducts. The extent and time of gonadal damage during foetal life influences the formation of the duct. In later life hormonal influences may further affect the duct derangement. A similar set of conditions may be applied to the development of the duct system of cases of purely gonadal damage as well as multiple deformities (Turner's syndrome etc.)

In the latter case gonadal disturbances must be considered as one disturbance amongst many others The genetic factor involves a further subdivision. Rarely do gonads lack an anlage three cases of agonadism have been published to date amongst which are two of my own (Overzier and Linden, 1956) Since there is no anlage, the stimulation of the gonads is found lacking The *Theory of Initial and Permanent Induction of the Gonads* (Overzier 1956a) guides one in the subdivision of the intersexual forms and enables a functional explanation of syndromes, which differ in appearance, but are in reality similar in development.

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DISCUSSION ON PAPERS 8 9 and 10

LENNOX There are two things I would like to ask about testicular feminization. First of all perhaps I misinterpreted Dr

Sachs and Danon's paper in the *Lancet* recently when I took it to indicate that these cases were not only XXY but would have the appearance of a female sex chromatin. Now I understand that everyone else who has sexed any of these cases finds them to have the male nuclear appearance. I would like to have a definite assurance from Dr Sachs that his cases also appeared to be male and if that is so how he reconciles it with his suggestion that their constitution is XXY? The other matter is that I think we ought to try to settle what we are going to call these cases. Dr Swyer in a letter to the *Lancet* complained about their being referred to as "testicular feminization of Morris", pointing out quite accurately that very many people had described cases before Morris and that all he had done was to give them a label. I think we ought not to use Dr Swyer's own name of "male pseudohermaphroditism", as surely there are many other varieties of male pseudohermaphroditism. It is an insufficiently exact name. This particular syndrome is one of very great interest and one that is probably going to have a good deal of attention paid to it in the near future. It is essential, therefore, to agree what it is to be called, so that people won't start talking about different things under the same name, or the same thing under different names.

SACHS: I want to thank Dr Lennox for raising these points, because there seems to have been some misunderstanding of the reasons why we suggested that "testicular feminization" may be XXY. Considering first the question of nomenclature, we have no particular preference for the term "testicular feminization" to describe this syndrome (in fact it is not even strictly accurate since the feminization does not seem to be induced by the testes) and we use it only because we feel that this very characteristic syndrome should have a specific name and that the name suggested by Morris is the most convenient one to use at the moment. If, however, anyone suggests a more appropriate terminology we would be quite ready to consider any suggested changes in nomenclature.

Regarding the interpretation of the sex chromosome constitution of this syndrome, it is clear that if one only examines a buccal mucosa smear or a skin biopsy by Professor Barr's method, one would conclude that the sex chromosome constitution is like that of a normal male, i.e. XY. But when in addition one also examines the skin for the proportion of cells with three and with two chromocentres in the young spinous cells, where one can detect individual chromocentres (and this cannot be done with buccal smears since these only contain the older cells) there is a difference. In the two cases that we examined we found a higher number of

cells with three chromocentres than in either normal males or females. We independently obtained the same result in both cases which came from two different families, and in neither case did we know at the time of the sex chromosome diagnosis the type of syndrome that we were examining In view of the finding that many young spinous cells had three chromocentres and for other genetic reasons that I will give later we therefore suggested that the sex chromosome constitution may be XXY

Now why do the old spinous cells give a diagnosis that one could interpret as XY? In order to answer this one has to ask the additional question why does an XX produce a clear double chromocentre in the old spinous cells in females and why does the single X not produce a single chromocentre in these cells in the male? The answer to this seems to be that male determining factors can inhibit the capacity of the single X to form a chromocentre in the old spinous cells of a normal male, so that one can assume that they can also inhibit the formation of a chromocentre in these cells by the two X chromosomes in an XXY

In addition to the evidence from the study of chromocentres, there is also the genetic evidence from the study of pedigrees The existence of pedigrees with this syndrome has been known for more than forty years, and it has been shown that if one adds the number of normal males, normal females and intersexes in these pedigrees, one finds a ratio of one female to one intersex plus male. Since one normally obtains a ratio of one XX to one XY it can therefore be assumed that the intersexes have a Y chromosome In addition, as Levit pointed out twenty years ago, since only families with at least more than one case of this syndrome will be included in a pedigree, there will be in the collection of the data a bias in favour of the number of intersexes and therefore automatically a reduction in the number of normal males. Making allowance for this bias in the collection of the pedigrees, it is possible to calculate that the ratio of normal males to intersexes may also be equivalent to a ratio of on to one The syndrome is transmitted only by normal females and one can explain the pedigrees in two alternative ways—either that the intersexes are XY and that there is a mutant gene that determines the inter sexuality or that they are XXY There is thus this genetic data that can give XXY as a possible explanation and the data from the study of chromocentres, that was arrived at quite indepen dently that can also be explained in the same way

SWYER I have no objection to the term "testicular feminization which I think is probably as apt a description as any I only objected to the eponym "Morris" being applied. If an eponym is

attached to a syndrome, it should be the name of the first person who gave an adequate description of the condition and I don't think that is true in the case of Morris

DAVIDSON In Professor Overzier's paper he suggested that the gonad determines the ducts, but one can get a normal testis with absence of the vas In such a case the vas must have been present if there are kidneys. The mesonephric duct must have grown down to reach the bladder and have given off its bud to produce the kidneys. We have got to consider therefore not only the development but also the possibility of involution In the condition of absence of the kidneys one does find evidence which suggests that the involution has taken place through the process given the name of Streeter's dysplasia for in the tissues there are signs of old hæmorrhage and calcification. In one case I found in a bit of the fallopian tube which remained calcification and hæmosiderin deposits suggesting that at one time there had been a paramesonephric duct but that it had undergone involution I think therefore that in addition to the possibility of the gonad determining the ducts we have got to bear in mind that involution of the ducts may have taken place. This may complicate the diagnosis in some cases and even indicate another mechanism in absence of the ducts.

JOST *May I add a few comments or questions? First concerning* "testicular feminization If as Dr Sachs suggested the syndrome results from a lack of sensitivity of the target organs to the testicular masculinizing secretions then the testis is not responsible for it. The expression 'testicular feminization which introduces an inadequate explanation should therefore be discarded In his very interesting paper Dr Prader suggested a lack of synthesis of hormones by the adrenocortical glands and the testes perhaps as a consequence of an enzymatic deficiency favouring the view that the adrenal cortex was not stimulated to produce corticoids by ACTH May I ask Dr Prader if he tried gonadotrophic hormone and if the testis released more androgens under such a stimulation?

PRADER No we did not try gonadotrophic hormone This was the first of the two cases we have seen—a boy the other was a girl—and the boy died long before we realized what was going on The ACTH test was not done on the girl We have thought about it and hope to do it in the next case. May I add one word about the name "testicular feminization If Dr Sachs is right in his hypothesis, and I think most people believe that it is a resistance of the end organ and not dysfunction of the testis, the right name would be an inborn androgen resistance that

would say just what is going on, but I think we do not really know whether this is true. A colleague of mine in Basel is just preparing a paper in which he contends that the testis is wrong He gave testosterone injections and could provoke the growths of axillary hair which is in contrast to the experience of Wilkins. I think there are not many patients where this has been tried. So long as we don t know what the condition really is, I think testicular feminization is a good name As soon as we know what *it really is then we must change it.*

JOST I would like to ask Professor Overzier one question. In the differentiation of the sex ducts he distinguishes two kinds of inductions produced by the sex glands an initial and a permanent induction. I did not exactly understand what is the effect of the initial induction on the sex ducts. I know of no animal experiment showing two successive inductions. For instance in Wolff's experiments, in which he castrates bird embryos, at very early stages, by x rays, he did not notice any initial induction. Concerning the sex ducts I prefer another conception. From the point of view of comparative embryology the Wolffian duct is first a renal duct—the mesonephric duct—established long before the gonad itself The Mullerian duct has also most probably some early relationship to the pronephros Thus the two embryonic ducts originally are not sexual structures. The Wolffian duct is later forced into the genital sphere by the testicular hormone which transforms a renal duct into a sex duct. This is a permanent induction.

OVERZIER We think it is impossible to show the point by experiment because the changes must occur too early It is only possible to castrate when the gonads are there to a certain extent.

BARR No one has mentioned the theory suggested by Witschi I don t know whether anyone here has the sort of biological experience to comment on this work—I certainly have not. His view based on experiments with frogs eggs, is that in gonadal agenesis, and perhaps in Klinefelter s syndrome the basic difficulty may be the fertilization of an over ripe ovum, and the lack of migration of germ cells from the extra embryonic entoderm of the yolk sac.

SWYER Apropos of this theory such evidence as there is in relation to mammals is that over-ripe eggs are probably not fertilizable. Certainly in rabbits the experience is that within six hours of ovulation the egg is no longer fertilizable and it is very likely that the same is true in the human. Of course this does not exclude the possibility it merely makes it less probable.

JOST In connection with the point raised by Dr Barr I should

like to mention one fact. Gonadal agenesis was recently experimentally produced in amphibians by Houillon. In the newt *Pleurodeles* it is possible to suppress more or less completely the differentiation of the mesonephros by stopping at very early stages, the caudal wards growth of the Wolffian duct, before it reaches the level where the mesonephros should differentiate In the absence of the mesonephros, no gonad develops The first anlage of the genital epithelium retrogresses and gonadal agenesis occurs. I don t know to what extent this result is valid for other animal species or for humans. But the possibility of a developmental correlation between the condition of the gonad and that of the mesonephros should not be overlooked

LENNOX Could anyone give any evidence as to the presence of Wolffian duct remnants in cases of Turner s syndrome?

SWYER In so far as the gonadal remnant contains a rete and the medullary canal, I suppose there are Wolffian duct remnants.

11

THE SIBS AND CHILDREN OF HOMOSEXUALS

ELIOT SLATER, M.D. F.R.C.P.
Genetics Unit, Institute of Psychiatry, Maudsley Hospital
London, S.E.5

About twenty years ago, stimulated by the work of Goldschmidt, Lang suggested that genetic intersexuality might play a part in the causation of homosexuality. He argued that, if some male homosexuals were genotypically female, then one should expect an excess of males in their sibs. He investigated the sex ratio of the sibs of 1015 male homosexuals and found a ratio of males to females of 121 : 100 (Lang, 1940). Similar investigations by Jensch (1941) of the sibs of 2072 homosexuals showed a sex ratio of 115 : 100. Both of these greatly exceed the expected figure of 105 : 100.

A direct test of Lang's theory was carried out by Pare (1956). He examined the chromosomal sex of 50 male homosexuals, and of 25 male and 25 female control subjects. He took mouth scrapings, stained them with hæmotoxylin and eosin, and examined them for the presence of chromatin spots, using the criteria suggested by Emery and McMillan. The slides were randomized before being examined, so that it was not known at examination whether they came from proband or control. The percentage incidence of chromatin spots ranged between 22 and 65 per cent in the scrapings from females, between 0 and 6 per cent in those from males, between 0 and 8 per cent in those from homosexuals.

It would seem, then, that the intersexual theory, at least in its simplest form, would have to be abandoned. The alteration of the sex ratio in the sibs of homosexuals remains to be explained. Some further confirmation of the existence of a shift might also be thought desirable.

For this purpose an examination was made of the case records of all patients admitted to the Bethlem-Maudsley Joint Hospitals, either as in-patients or as out-patients, from the 1st January 1949, to the 30th June, 1956, inclusive, who had been classified diagnostically as the subjects of sexual deviation (320·6 of the

International List of Diseases and Causes of Deaths) These persons included 503 males and 45 females The males comprised 286 homosexuals, *116 exhibitionists, 18 transvestists, and 83* others the females 39 homosexuals 6 others The case records of four groups were more closely examined (MH) male homosexuals (Ex) exhibitionists, (Tr) transvestists, (FH) female homosexuals and the principal data are given in the table below

	MH	Ex	T	FH
Mean age on admission	3 7	3[illegible]	3 4	3
Mean age of mother at birth of patient	3 6	[illegible]8 7	[illegible]8	[illegible]9 9
Mean number of sibs	3·07	[illegible]·7[illegible]	6	·95
Mean position in sibship	·68	0·47	0·6	0·55
Proportion of males in sibs	0·53	0·43	·6[illegible]	·55
Proportion of patients married	·13	·66	·6	[illegible]
Mean number of children per person married	·4	·8	[illegible]7	0·[illegible]5

There are no significant differences between the mean ages on admission, so that we need not expect the other statistical data to be influenced by the four diagnostic groups belonging to different age groups.

The mean age of the mother at the birth of the patient is significantly higher for the male homosexuals than for the exhibitionists, who are the only other group of comparable size the difference between the means, 2·88 years is over four times its standard error Looked at in another way 81 out of 240 male homosexuals were born of mothers of 35 or over 15 out of 100 exhibitionists the value of χ^2 for this distribution is 12·25 for 1 d.f., with $P < 0{\cdot}001$ In the cases of 46 homosexuals and 16 exhibitionists, the age of the mother at the birth of the patient was not known. It is possible that a cultural factor underlies the difference. The exhibitionists probably came from a lower social class than the homosexuals, which would affect the mean age at marriage of their mothers

The relatively high mean number of sibs of the male homosexuals would go against any suggestion that there was some factor tending towards infertility in the family background It is also to be noted that, though these homosexuals rarely married, those who did produced a normal number of children.

The mean position of the proband in the sibship was calculated from the formula $\left(\sum \frac{m-1}{n-1}\right)/N$ in which n is the number of

persons in the sibship where the proband is *n*th in order of birth, and *N* is the total number of sibships included in the calculation. The expected value of this statistic is 0·5. It is seen that the homosexuals arrived conspicuously late in order of birth. In this respect they are comparable with twins and with mongols. The mean position in the sibship of a series of psychiatric twins (Slater 1953) can be calculated from the material there provided as 0·69; from Penrose's data on mongolism (Penrose 1934) a figure of 0·79 can be obtained.

Another method of examining birth order may also be used. Whatever its size, *s*, as defined above, can be divided into 5 equal parts or quintiles, when *n* will either lie wholly in one of them, or be fractionally distributed between two or three. In this way the data for all sibships, whatever their size, can be summed. Dealt with in this way, the data gave 37·2 homosexuals in the first quintile, 34·0 in the second, 36·0 in the third, 51·4 in the fourth, and 64·4 in the fifth. The order of birth of 63 homosexuals was not known. These figures show an excess in the last two quintiles which is highly significant, χ^2 15·29, 4 d.f., $P < 0·01$.

We must now consider whether the late placing in birth order is to be accounted for by the greater age of the mother at the birth of the proband. Fisher's method described by Penrose (1934) may be used. Unfortunately, in applying it to this material, there was a large loss of information: both birth order *and maternal age have to be known in the same case* but either or both were unknown in a high proportion of the larger sibships. The quintile distribution corrected in this way gives the figures 30·2, 27·2, 24·4, 38·8, 47·4. There still remains a tendency for the probands to come late in birth order, but it is no longer significant: χ^2 6·06, 4 d.f., $P = 0.20$ app.

The male homosexuals had 362 male and 325 female sibs: the ratio of 111 : 100 is only a little below that found by Jensch, but does not differ significantly from 105 : 100 owing to paucity of numbers. It is, however, worth noting that the exhibitionists had an excess of female sibs, 109 : 144, and the difference between the homosexuals and the exhibitionists is significant, χ^2 6·59, 1 d.f., *P* only slightly exceeding 0·01.

It has been suggested that a possible explanation of an excess of males in the sibship of male homosexuals might be that the fact of having a brother tended to cause homosexuality: for instance, mutual sex play occurring with a brother during childhood might have a predisposing effect. If this were so, one might expect that it would be the sibs closest in age who would have the greatest psychological effect. However, *a count of the sex distributions of*

next older and next younger sibs showed no greater male preponderance than that obtaining in the totality of sibs

The male homosexuals had 23 male and 28 female children, where the exhibitionists had 48 male and 38 female children. It is interesting that the sex preponderance in the sibs is reversed in the children in both cases. But the numbers are too small to have any importance

CONCLUSION

As the result of our study we find a male preponderance in the sibs of male homosexuals, confirming the earlier work of Lang and Jensch but smaller in degree, and in this material not statistically significant this leaves the problem of the existence of such a preponderance and its explanation where it was In addition, it has been found that, compared with exhibitionists, homosexuals tend to be born of older mothers, and are also born late in birth order the latter effect possibly being accounted for by the former

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DISCUSSION

HUGGETT The point about the birth to elderly mothers links up with the incidence of mongolism which also tends to occur with elderly mothers

SLATER Yes, indeed—I think the interest of these findings is that, if they were confirmed, they would bring homosexuality into line with these other conditions like twinning and mongolism.

HUGGETT Apropos twinning—is that a substantiated point? I don t think that it has been shown that arrival late in a sibship or the age of the mother is responsible Professor Penrose may know about this

PENROSE As far as I know it is the maternal age and not the birth order which affects twinning

SACHS Is it known whether there is anything peculiar in the finger-print patterns of homosexuals as there is in the finger print patterns of mongoloids?

SLATER I am afraid we have not looked at any finger prints.

GREENE Isn't there a much more likely explanation of this difference in the age of the mothers? By the time the mothers are getting old they are getting rather tired of their husbands. They therefore attach their affections more to their sons and in that way perhaps acquire that undue power over them which is usually associated with homosexuality

12

TRANSVESTISM

C. N ARMSTRONG M.D F.R.C.P D.P.H
Lecturer in Clinical Medicine, University of Durham

To open a discussion on transvestism I intend to describe a very typical case who consulted me recently He has been fully investigated and illustrates the main features of this syndrome.

Male aged 28 years occupation—cook.

HISTORY

Father jovial man with artistic gifts, who died at the age of 45 from a cerebral tumour when the patient was 15. Mother unemotional and cold type of woman. Patient, third in a family of four—two sisters and one brother

As a child was healthy and free from neurotic traits He had a detailed memory for his early life and would appear from an early age to have a strong preference for girls games and chose girls as his playmates Already at the age of three or four he had begun to dress in feminine clothes on every possible occasion despite strong disapproval from his parents, brother and sister He would go to school dressed as a boy and as soon as he arrived home would change into girls clothes. On leaving school he worked for a year at a corset factory and then was apprenticed to a piano tuner with whom he worked for five years. At this time he adopted certain feminine features in his dress to which his mother made very strong objections, and he experienced cruelty and ridicule from society on account of his femininity During the past four years he has worked as a waiter and chef.

He has always identified himself with girls and the feeling that he is a woman compelled by society to live and act as a man has dominated his life. His intense belief that he is essentially female issues partly from the fact that he is convinced he has never had to make conscious efforts to imitate female gestures and manner isms or affect an interest in feminine activities. These things have come to him with a spontaneity of instinctive attributes. He expresses hatred of and revulsion from the outward signs of masculinity in his sexual organs and considers he has a right to have them removed so that he can approximate more closely to

feminine anatomy and can live as a woman without a feeling of deceit that is repugnant to him, or a fear of discovery and persecution. At present he is *denied all possibility of friendship* whereas if he were not so indubitably male he would be able to achieve platonic relationships with either men or women.

He has never had desire for normal or perverse sexual intercourse, but several times he has become romantically infatuated with virile men. His love for these men has been intense and deep but of a purely platonic nature The thought of sexual physical contact with men or women disgusts him. Yet he has indulged in fantasies of pregnancy and has a passionate desire for motherhood.

His left breast was removed nine years ago in Queensland Australia, but I have been unable to obtain any information as to the pathology About three years ago he began taking œstrogen in a total of three years, starting at the age of 25 he took somewhat intermittently twenty bottles each containing 100 50 mgm tablets of hexœstrol.

He always wanted to dress as a female and never wanted to wear a suit or tie. He has never grown much hair on his face but this has never really been a problem to him.

On clinical examination the patient's physique was slender and graceful, his skin smooth and almost wholly hairless his face mobile and expressive. His gestures and mannerisms, as well as his facial expression were feminine in what strikes one as a spontaneous and unaffected manner His weeping which was witnessed a number of times at interview was feminine too in the high pitch of the cry the abundance of tears the prolonged loss of control and the changes of expression. Penis and testicles were small—the right had on it a hard nodule and there was a sinus opening on the scrotal skin to which it is adherent. Facial hair was definitely deficient for male standards at 28 years of age and pubic hair although tending to umbilicus, was below standard. There was gynæcomastia on the right side the left breast had been removed for a growth at 21 years of age. His general appearance was undoubtedly rather feminine (Figure) No abnormal signs heart, lungs, abdomen, or central nervous system Fundi normal. Blood pressure 155/100

Oral mucosa smears from both cheeks were found to be chromatin negative Examination of 400 polymorphs did not show anything resembling a female club

Personality He would appear to be a kind tender and gentle person with a strong craving for friendship. But his disability has made this difficult as he repels most men and has only rarely

been able to establish a satisfying friendship on a platonic basis with women He lacks any capacity for combat or assertion even in circumstances where he has been victimized and exploited. He is subject to strong feelings of inferiority which he attributes to



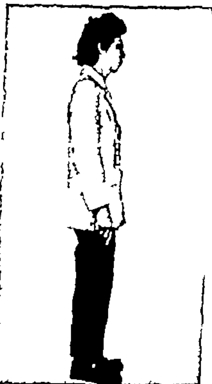


FIGURE. Male transvestite The left breast had been removed some 7 years before.

rejection he has suffered from society He is hard working conscientious, and methodical in all that he undertakes, but is not preoccupied with order nor is he rigidly tied to routine. He aims high in anything that he undertakes and his behaviour is governed by strong moral principles

The general impression is of a person of intelligence and integrity

The *Terman Miles* masculinity femininity test yielded a score of 114 which is well over towards the female end of the female range of scores. The pattern of responses is in other words, more feminine than that found by this test in the majority of females.

Testicular biopsy There was a sinus with a small aperture in the skin at the most dependent part of the scrotum at the right side. It entered the epididymis and appeared continuous with the vas deferens. There was no evidence of any inflammatory disease process. It was thought that this sinus was a seminal fistula Histologically it showed a subcutaneous sinus and intense non specific chronic inflammatory change

Histology of specimens from both testes show atrophic testicular tissue No evidence of spermatogenesis Scanty interstitial cells. Marked fibrosis and thickening of the basement membrane of the seminiferous tubules.

Urinary 17 ketosteroids 1 3 mgm in 24 hours.

	Pregnanediol (mg/24 hr)	Œstriol	Œstrone (μg/24 hr)	Œstradiol	17 KS	17 Ketogenic S mg/24 hr
Feb.	6	4 8	7	4 8	7	
	5	5 4	4 5	4 8	3	3 5
3	7	3 7		—	6	3 6
4	6	3 3	3 5	9	4 8	

The patient obviously has poor testicular and adrenocortical functions. Œstrogens, with the exception of oestradiol which is above normal, are normal to high normal for a male

COMMENT

1 Transvestism, also named "Eonism" after the Chevalier d'Eon de Beaumont who was a diplomatic agent of Louis XV and was taken to be a woman but proved at post mortem to be a normal man is clearly an inaccurate description of the personality in such cases and is characteristic of this patient. The preference to wear feminine dress is only one expression of a strong desire to become identified with women and to be regarded as female by society Transvestism in the sense of longing to wear the garments of the other sex may not even be found although the majority of cases do have this desire and the great majority of transvestists who come under investigation in clinical practice also manifest the syndrome described here. It is relatively common and some authorities judge it to be second in frequency only to homosexuality

2 Transvestism may be symptomatic of homosexuality and occasionally of other sexual perversions, but only a small minority of homosexuals are transvestists And transvestism as applied to

cases such as that described i.e. eonism, is a phenomenon quite distinct from homosexuality The two are frequently confused even by psychiatrists and psycho-analysts but there are the following clear lines of distinction

(a) The crucial characteristic of the homosexual is the desire for a physical sex relationship with a person of his own sex. The eonist (who is more often male than female) is repelled by the physical aspects of a homosexual relationship Rarely the eonist, as a result of his platonic love for a man, becomes transiently involved in a physical relationship with him His usual response is one of disgust and revulsion. *Homosexuality and eonism may* therefore coincide but the association is rare and the two phenomena are distinct. If an active sexual life is led by an eonist, it is more often a heterosexual one

(b) Homosexuals do not as a rule want to change their sex and identity This is the fundamental anomaly in eonism.

(c) A conspicuously feminine appearance and a lifelong preference for feminine games and activities are far more common in eonism than in homosexuality although of course they are sometimes found in the latter

(d) From all the detailed case studies published it would appear *that the preference for the feminine role in eonism is evident* from early childhood Such early manifestation is unusual in homosexuality

(e) The fantasies of pregnancy and the passionate longing for a maternal role, together with the desire for castration in an attempt to achieve anatomical resemblance to a woman are characteristic of eonism and so rare in homosexuality that cases of the latter in which they occur may be regarded as suffering from two independent syndromes.

3 The aetiology of the condition is at present obscure. Cases published so far in men have been chromosomal and gonadal males. Psycho-analysts have attributed the disorder to a castration complex but to this is also attributed most other sexual perversions so that this is not very helpful. There is a tendency for the mother to be an abnormal personality as in this patient's family and for there to be a predominance of feminine influence in the family the father being submissive or insignificant, the brothers presenting an example in personality pattern that is repellent and frightening However there has been no systematic study to ascertain whether such features are found consistently Moreover this predominance of femininity is also described in a proportion of families of homosexuals and of course, in the families of individuals who have achieved normal sexual adjustment.

It is possible that such psychological factors make some contribution, but in a phenomenon manifest so early in life and in which such strikingly feminine characteristics of physique are manifest as in this case a constitutional factor probably on a genetic basis is likely to be the specific one There is no doubt in this case there is physical deficiency of masculine characteristics and I feel confident the limited period he took œstrogen cannot be held responsible. It is highly probable that the amputation of the left breast four years before he took œstrogen was for gynæcomastia.

4. TREATMENT

No treatment has so far been found to be successful in fundamentally altering the attitude of the conirt, but psychotherapy can frequently help him to a better adjustment.

SUMMARY

1 A typical case of male transvertism is described. He has shown a strong preference for a feminine role and female dress since early childhood. Genitalia are male but appearance very feminine owing to scanty facial hair feminine stance and build as also facial and emotional expression There is right gynæcomastia. His personality is gentle submissive, sensitive and artistic. Psychological testing reveals a markedly feminine disposition.

2 Endocrine tests reveal deficient testicular and adrenocortical function. Testicular biopsy shows absence of spermatogenesis and scanty interstitial cells a left seminal fistula was present. Examination of oral mucosa and of polymorphs shows him to be chromatin negative

3. It is stressed that the condition typified by this case goes well beyond a mere desire to wear the attire of the opposite sex. The quest for female identity is pursued with fanatical zeal. There are fantasies of pregnancy and of a maternal role. Œstrogens have been self administered and amputation of genitalia sought.

4. There is a tendency to romantic infatuation with virile men, but no desire for a physical relationship The distinction from homosexuality and other perversions is stressed.

5 The ætiology of the condition is obscure Psychological factors probably contribute in causation, but the specific factor is likely to be a constitutional one on a genetic basis.

(*I should like to express thanks to Professor Martin Roth for his help*)

DISCUSSION

BISHOP May I ask whether you considered that the gynæcomastia here was due to the œstrogen given or do you think it was a spontaneous gynæcomastia?

ARMSTRONG I have considered that point. The left breast was removed for some swelling but I have not been able to find out why The patient says the swelling of the right breast came up after he had taken œstrogens

BISHOP I have had one or two cases with gynæcomastia and I have also had cases where physically the individual was a very virile male with no suggestion of any feminine traits. I would agree with you that the problem is to know what to do with these people From the experience I have had and I have had considerably more experience of them than I would have liked to have had, it is extremely difficult to know what to do with them. The plastic surgeons won t touch them and the psychiatrists, very often are completely uninterested in them. I have found psychiatrists who regard them as perverts, schizophrenics, and so on. The one thing I do feel is that these cases are absolutely genuine in their feelings and are really very tragic. I think it would be a very good idea if the psychiatrists plastic surgeons, and endocrinologists got together and really tried to think what is the right thing to do with these people

SIEBENMANN May I ask if this testicular biopsy was taken after œstrogen treatment too because it shows typical tubular fibrosis.

ARMSTRONG Yes.

STEWART Clinically I would say this case was very similar to a chromatin negative Klinefelter s syndrome In carcinoma of the prostate when œstrogens are used you get the gynæcomastia but to the best of my knowledge there is no change in the distribution of pubic hair The photograph shown here showed a female distribution of pubic hair

LENNOX But that was not, surely the biopsy of a Klinefelter s syndrome even after œstrogen treatment?

FERGUSON-SMITH Were the urinary gonadotrophins measured? One would expect them to be low

ARMSTRONG I was not able to get them measured.

GREENE I would like to know whether Dr Armstrong considered the possibility that his patient was suffering from a hepatic deficiency I saw not very long ago a very tough, rugger playing hard drinking happily married man who rather rapidly

developed gynaecomastia associated with what you might call an acute transvestism. During the day he was conscious of wanting to appear as a woman which disgusted him, and during the night he dreamed fantasies of being a woman He went to his doctor who decided to treat him with testosterone injections When he had had three injections of 100 mg his breast tissue began to subside and his transvestism entirely disappeared He was sent to me and I could find no evidence of anything the matter with him except slight remnants of his gynaecomastia and he assured me that the mental symptoms had disappeared entirely In view of the fact that he had gynaecomastia I went through his liver function tests and established beyond all doubt that he was suffering from cirrhosis of the liver And it seems that both his mental symptoms and his gynaecomastia were due to a failure of the liver to conjugate his circulating oestrogens.

ARMSTRONG The symptoms in my case went back to the age of three.

BISHOP In my experience practically all these patients have been treated with androgens at some time or another without any effect at all.

RIIS We try to help these patients because they lead a horrible life and often commit suicide because they feel themselves outside the social community Plastic operations are not illegal in Denmark and have been done in several cases with I suppose as good results as you can expect unfortunately since one patient thereafter went around the world and showed himself in night clubs, the doctors concerned have been unwilling to do any more

SWYER I want to make one small point about transvestism and that is that it is not confined to the male sex. There are female transvestists who wish to be turned into males and they constitute at least as great a problem to the individual as does the male who wishes to become a female.

OVERZIER I don t doubt Greene s case of liver damage of course but in the cases of transvestism wh ch I have seen there was no liver damage. All my cases have been sexed and shown to be genetic males including two castrated more than 20 years ago

DAVIDSON There is one similar report (Witschi and Mengert, 1942) in which t might be argued that in fact the patients were true hermaphrodites for there appear to be ova in the walls of the testicular tubules. It is always very difficult to be absolutely certain we haven t missed ovarian tissue even with histological studies. We have seen one very interesting case—one of our first cases of the true Klinefelter s syndrome, who gave practically the same history as Dr Armstrong s case His mother was a most

unsatisfactory person, a drunkard who disappeared from home for several months at a time As a boy he had always been called "Sissy" and eventually when he left school he drifted through various employments and the only ones he held for any length of time were of an artistic nature He turned up in London after having been in trouble with the police through his associations with men and he came to our psychiatric department dressed as a female We examined his blood and found that he had drumsticks in the leucocytes. He looked a female assumed female attitudes and had even got a wide carrying angle, but he had well formed male external genitals apart from the small size of his testes Further examination including a testicular biopsy confirmed that he was a case of the true Klinefelter's syndrome He was in a dreadful psychological state could not fit into life at all, and had tried to commit suicide. He appears to represent another variety but of course he might also be a true hermaphrodite. One cannot section the whole of the gonads.

SIEBENMANN We have had one case of Klinefelter's syndrome with heterosexual, that is female, trends and curiously enough he was one of our two cases showing spermatogenesis.

LENNOX I think we should consider the problem of what one could say in a Court of Law as to the true sex of a person whose nuclear sex was at variance with the sociological sex.

BISHOP One of my cases of transvestism is also a Klinefelter with female genetic sex, which raises the point Dr Lennox has mentioned. He spends his time in and out of gaol When he comes out he goes straight off to Leicester Square and importunes and is sent back to gaol for having homosexual relations. In fact, of course, he is a genetic female, which raises a rather interesting legal point.

ROBERTSON SMITH We invited a legal authority to the meeting to discuss this type of problem but unfortunately he was unable to come. Perhaps we may be able to arrange this at some future meeting

THE NEUTROPHIL SEX NODULES IN KLINEFELTER'S SYNDROME

W. M. DAVIDSON, M.D. and
D. ROBERTSON SMITH, M.A., M.D.
King's College Hospital Medical School, London

Excluding the so-called "false type", a heterogeneous collection of cases due to a variety of causes, Klinefelter's syndrome can be defined as a condition in which individuals of a male phenotype have chromatin positive nuclei.

They are complete males apart from the small size of the testes, the resulting sterility and sometimes a partially feminine development of the secondary sex characteristics.

The defect responsible for this strange "chiasma" must occur before the parting of the ways in sex development and the individuals could be considered as either males with female cells or *females with male genital organs.*

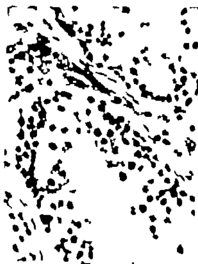
The condition does not appear to be associated with any specific change in the appearance of the individual, and even in the cases with a more feminine development of the secondary sex characteristics the level of oestrogen excretion is not raised and is often low even for a male.

The characteristic change in the testicular tissue, a hyaline degeneration in the tubules, and a nodular aggregation of the interstitial cells is also not completely specific. In most cases there are in addition areas in which the tubules retain the Sertoli cells, especially where the tubules are embedded in masses of interstitial cells, and even areas of complete spermatogenesis may be found as in case Mr W. (Fig. 1). At the other extreme there may be almost nothing left but masses of interstitial cells. So far there has been no evidence that there is any close correlation between the testicular histology and the hormone excretion levels and the gonadotrophins have been high even when there was some active spermatogenesis.

A curious feature is the low frequency of the characteristic drumsticks in the neutrophil leucocytes (Fig. 2). *Using the method of counting the neutrophils until six drumsticks were* found, in seventeen cases of Klinefelter's syndrome the mean



( )



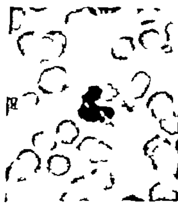
(*b*)

FIG. ( ) Testicular biopsy in one case of Klinefelter syndrome (Mr W.) showing on the right the ypical tubular atrophy and on the lef an area of spermatogenesis. Masses of Leydig cells lie between the tubules. (H & E. 60.)

FIG. (*b*) High power view of the area of sperma ogenesis. (Iron hematoxylin 240.)



( )



(*b*)

FIG. 2 Characteristic neutrophil drumstick in blood film from ( ) normal female and (*b*) case of Klinefelter syndrome (Jenner-Giemsa ×930.)

difference between the actual number of neutrophils that had to be counted and that anticipated from the average lobe counts was greater than twice the standard deviation. This relationship was still present when the drumstick frequency was analysed for each

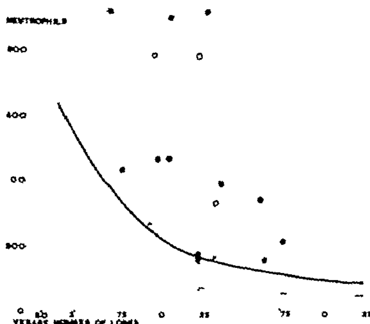


FIG. 5. Graph showing the number of neutrophils counted to find six drumsticks plotted against the average number of lobes per neutrophil in 550 films from "normal" females (and continuous line). The interrupted lines represent the standard deviation. The findings in 7 cases of Klinefelter's syndrome are indicated by O

neutrophil lobe group (Figs. 3 and 4). No such change in frequency has been found in true hermaphroditism or female pseudohermaphroditism.

Up to the present no similar diminution in the frequency of the sex chromatin nodules in other cells has been recorded but we have only found infrequent nodules in the Leydig cells.

A number of possible explanations for this diminished frequency include

(a) A neutrophil shift to the left. This has been excluded and in any case would have been allowed for as the average lobe count was taken into consideration.

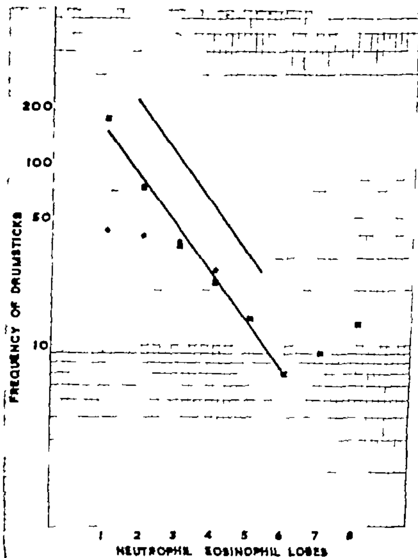


FIG. 4. The findings from 950 films from "normal" females analysed to show the number of neutrophils per drumstick in each lobe group of neutrophils (and lower line) and eosinophils (C). The neutrophils in 7 cases of Klinefelter' syndrome are shown (● and upper line). The numbers of eosinophils found were too small to consider

(*b*) A form of chimaerism with an admixture of male and female cells. Such a state has been found in human twins and has been created artificially in rabbits. In the latter we used X-irradiation to remove the marrow in a male rabbit and then grafted female marrow. When this has taken a race of female leucocytes appear in a male rabbit (Fig. 5). In the Klinefelter cases there is no support for this explanation for the individuals were not twins and there was no evidence of mixed blood groups.



FIG. 5. One of numerous "female" amphophil leucocytes from male rabbit after total body irradiation and successful grafting with female marrow. (Joiner-Gleeson 1000.)

(*c*) The male and female elements might have arisen through a mosaic condition. If this were the case similar changes should have been demonstrable in the cells of the other tissues and this has not been recorded. A partial mosaic condition limited to the myeloid tissues is still possible and were the individual to have the abnormal genotype XXY the marrow cells *might* separate into XX and XY.

(*d*) Another possible explanation of the low frequency is that it is caused by the factor as yet unidentified, responsible for the reduced number of drumsticks found in a small proportion of apparently normal women.

Several theories have been put forward to account for Klinefelter's syndrome. It has been suggested that the individual might have the genotype XXY. In this case the phenotype should be female not male and as the mother might be expected to be of the genotype XXX her neutrophils and other cells should have enlarged masses of sex chromatin. The drumsticks in the mother of a case of Klinefelter's syndrome have been found to be normal.

Another and possibly the most likely explanation is the occurrence of a mutation similar to tra described by Sturtevant which caused the formation of male gonads and genitalia in the female drosophila. The preponderance of sisters 12 sisters to 5 brothers in our cases although suggestive, is far from proving that the affected individuals bear the Y chromosome

A final possibility seems to be that the condition arises from a separation of the sex chromosomes from the factor responsible for the sex chromatin nodules. Although normally these two must be at least closely related we have not as yet any direct proof that they are identical Such a separation would be expected to affect all the sex chromatin and would not explain the low frequency of the drumsticks in most of Klinefelter's cases

Although so far no definite conclusion can be drawn as to the cause of Klinefelter's syndrome it does provide a powerful stimulus to us to contemplate the origin of sex in the development of the human organism.

(*We have to thank Dr J F Fowler Principal Physicist for his help in the experiments*)

DISCUSSION

SACHS Dr Davidson did I understand you to say that you had actually found mosaics in the blood groups of these individuals and if so were these associations of O with A or O with B or was there any regularity in the phenomenon?

DAVIDSON No I must not say we found a mosaic condition in Klinefelter's syndrome A possible explanation of the low frequency of the nodules could be that in fact one has a mixture of male and female nodules, comparable to the chimera type but there is no evidence of twinning in this syndrome Another possible explanation would be that it arose as a mosaic, but we have no positive evidence of that and against it we have the failure to find any such variation in the skin nuclei such as one would expect in a mosaic, unless the condition was limited to the bone marrow which I think Dr Ford suggested was unlikely to occur

SERR With regard to the radiation animals, I think it has been shown recently that the injected marrow is replaced towards the end of some months to a year by the original bone marrow element. Did you find this?

DAVIDSON No we have not found that so far Maybe because we have not had animals kept alive long enough, but during the time we have watched them the percentage of foreign cells does not appear to have dropped which suggests that the animals are

still utilizing cells originating from the transfused cells and there is plenty of bone marrow in the femora when we have killed them off

FORD If I may make a comment on experience with these mouse chimeras, the replacement of the grafted tissue by the cells of the host occurs in some combinations but not in others. In some the injected cells give a grafted tissue which persists indefinitely depending on the specific combinations in the mouse It may of course, be different in the rabbit.

DAVIDSON Possibly it depends on the degree of irradiation too

FORD Yes, certainly

DAVIDSON These rabbits had an adequate dose to destroy their own marrow and immunity mechanism.

FORD Yes, if you have less then you get very rapid replacement of the donated tissue by recovering tissue of the host.

SACHS Was this an inbred strain of rabbits? Was there any relationship between the donor and irradiated recipient?

DAVIDSON Yes and no For this particular one we simply took males and females from our own stock which is naturally inbred to some extent. We have also done it with other markers where there was much less inbreeding

SACHS Even so is it not an extremely interesting phenomenon that it should persist so long?

PLATT Has anyone tried to count the male and female sibs of cases of gonadal dysgenesis with male sex to see if they fit in with the expected ratio or not? If nobody has done this on an extensive scale could not we here between us do this quite easily I have seven such cases. I know Dr Polani has collected a large number and amongst us we must know a lot of them if you think it is worth doing The other question I want to ask is what is the cause of gynaecomastia in Klinefelter's syndrome?

POLANI I am very grateful to Professor Platt for suggesting pooling information on the sibships. Human sibships are as everybody knows, terribly tricky things and if you start selecting them and then further restricting the observations in other ways, you end up by having so many corrections that I think you cannot utilize the material statistically We must have large samples to get our information.

ASHLEY One further possibility as far as haematological sexing is concerned, is that the satellite body is a sex characteristic rather than a sex chromosome Dr Davidson has mentioned that certain cases of Klinefelter's syndrome are of female nuclear sex when the skin is examined but that the number of satellite bodies

seen on the neutrophils is lower than would be expected. There are also patients with the converse syndrome—Turner's syndrome—in which the skin sex is male but in which a female distribution of satellite bodies on the polymorphs is seen. I personally have seen two—in one we counted 6 satellite bodies in 360 cells, and in the other in one film we counted 6 satellite bodies in 60 cells. In both cases the sex of the cell of the buccal mucosa was male. Similar findings were mentioned to me in a letter from Professor Greenblatt who is reporting them some time this year. It does tie up with the work which I reported earlier this year of finding sex chromatin nodules in cells of the bone marrow including polymorphs, and finding the bump of chromatin which we recognise as sex chromatin in a series of polymorphs also showing satellite bodies.

PRADER. The same discrepancy between the buccal smear and the blood was observed and published by Kosenov in Germany some time ago.

BISHOP. Can anybody answer Professor Platt's question—what is the cause of gynæcomastia in Klinefelter's syndrome?

SIEBENMANN. I can't answer the question but perhaps I could add some pathological observations which perhaps add to the ætiology. In 20 cases of true Klinefelter's syndrome we have observed gynæcomastia 15 times. It occurred at any age, and there was no relation to the gonadal histology or to androgen deficiency. The only correlation was between gonadal histology and the F.S.H. excretion—we found the F.S.H. excretion not elevated in those cases where a lot of tubules were still patent, but when they were all sclerosed the F.S.H. was always raised. Only one patient had an elevated œstrogen excretion and a normal F.S.H. This patient had gynæcomastia. There is a probability that gynæcomastia is a result of an imbalance of androgens and œstrogens in these patients, the Leydig cells probably producing both.

BISHOP. I think that is what we felt too, in fact we are investigating these cases of Klinefelter's syndrome with gynæcomastia from the point of view of 17 keto-steroid excretion and œstrogen excretion in the urine.

LENNOX. Are there any conditions in which the F.S.H. is raised in which gynæcomastia is not common? A possible explanation may be found in the pituitary's apparent habit when it is producing one hormone in excess of also giving a slight rise in the neighbouring hormones, just as the melanophore stimulating hormone rises when the ACTH goes up. May not this be a similar phenomenon?

BISHOP What about the male climacteric? You don't get gynaecomastia in the male climacteric do you, and there are a few cases of elderly men with a raised F.S.H

LENNOX Are the levels comparable?

BISHOP Some of them are fairly high.

FERGUSON-SMITH May I add another impression about gynaecomastia? Many of our cases of Klinefelter's syndrome showed gynaecomastia, what has been described as a false gynaecomastia with adipose tissue only Our two cases which showed true glandular hypertrophy of the breast were both young 18 and 23 and they had high F.S.H.s. Going back into the history of our other cases I asked each one if he had ever had any swelling of the breasts and one case a chromatin positive individual, said he had. True gynaecomastia has appeared in our young cases and only false in the old patients

KLINGER I would like to ask Dr Davidson one technical question If on the same smear in which you get an abnormal count you repeat the procedure until you get 6 drumsticks—do you get the same result within a reasonable experimental deviation?

DAVIDSON We have done many repeated counts and of course you get a scatter round a point. Many of the points were the average of several counts. In the graph (Fig 3) two cases of Klinefelter's syndrome gave points near the line These again were derived from several counts of which an odd one fell below the line In the other Klinefelter's syndrome cases the limits of the scatter were well above the anticipated level for what that is worth

THE RESULT OF HÆMATOLOGICAL DETERMINATION OF THE GENETIC SEX IN DISTURBANCES OF SEXUAL DEVELOPMENT

H R WIEDEMANN Prof. Dr med.
Chefarzt der Stadtische Kinderklinik,
Krefeld, Germany

My co-workers present at this meeting Dr Romatowski and Dr Tolksdorf who have mainly carried the practical load of this work, and I feel very modest in this scientific circle, but as Schiller says Wenn die Könige bau n haben die Karrner zu tun

As far as we know my associates and myself were among the first to test and confirm the admirable discovery of Davidson and Smith

Since 1955 we have tested and applied the hæmatological method of nuclear sexing on material sent to us by many interested persons Although we are not able to tell you anything new or publish extraordinary discoveries, I hope that our results checked on a large number of sexually pathological cases, and presented in tabular form, may attract your attention.

We are able to confirm the value of the hæmatological method of nuclear sex diagnosis and for the first time give figures on sexually pathological cases in Europe on a large scale which compare with those so far only published in the United States by Money Wilkins and co-workers, and Nelson

Let me say this in advance Everyone should apply the method of nuclear sexing which he is used to None of today s methods is without fault.

Compared to the skin-cell nuclear investigation in different cell layers of Sachs and Danon the hæmatological way does not give such specific and detailed results, but it can only be of advantage in the present stage of these investigations to use several methods at the same time, one of them being the hæmatological method to advance one s knowledge. Everyone agrees that such com parative studies made on a large scale in co-operation with

different institutions, as has been done in the past with other studies, should provide further interesting and advanced knowledge

It should be mentioned that we adhered to the classical method of Davidson and Smith in our hæmatological examinations as has for instance Riis in Denmark and v. Harnack and Strietzel in Germany and contrary to other authors. Without the proof of at least six undoubted drumsticks no diagnosis of the femal sex can be made. Naturally the number of 500 neutrophil leucocytes could not be taken always as the absolute upper limit during cell counts if only one or two probable drumsticks were found in the number of 500, the cell count had to be continued

In Table I you see a summary of the hæmatological examinations for the diagnosis of nuclear sex made at my clinic in a period

TABLE I

HÆMATOMORPHOLOGICAL "NUCLEAR SEXING" IN ERRORS OF SEXUAL DEVELOPMENT

Number of specimens referred from other institutions 440

Surgery, internal medicine, urology pathology	63	Western Germany (including Saarland)	4
Psychiatry	48	Eastern Zone	44
Obstetric-gynæcology	66	Netherlands, Austria, Spain, Sweden	5
Pediatrics	245	Switzerland	67

(In addition 5 cases from our own clinic)

True hermaphrodites		5
(Nuclear sex ♀	)	
(Nuclear sex ♂	3	
Male pseudohermaphroditism (sensu communis		77
(Including "testicular feminization	20)	
Gonadal dysgenesis (sensu communis		85
(Nuclear sex ♂	66)	
(Nuclear sex ♀	7	
Female pseudohermaphroditism (sensu communis)		77
(Congenital adrenal virilism	70)	
(Female pseudohermaphroditism (sensu strictiori = without adrenal disorder)	3)	
"Klinefelter" syndrome		4
"Constitutional homosexuality" including Transvestism		38

of two years. It might be of interest to notice the different specialists who have sent us their material. Most of the specimens from Switzerland came from *Dr Prader (Zürich)* and *Dr Hauser* and Prof. Wenner (Basel)

Th constantly increasing amount of this material demonstrates

impressively the relative frequency of cases of the intersexual states and other errors in sexual development and indicates furthermore the considerable need for additional diagnostic help as well as wider knowledge in this difficult field of biology and medicine The ratio of 5 true hermaphrodites to 154 pseudohermaphrodites shows once again the great rareness of the true hermaphroditism, and the equal number of female and male pseudohermaphrodites 77 of each demonstrates once more the frequency of misdiagnosis in the past

The following table, Table II will show you the reliability of the hæmatological method of nuclear sexing executed by special ists In the first line are the undoubted cases of male and female pseudohermaphroditism of all ages. Naturally all these cases were diagnosed blindly (that is to say without knowledge of the clinical or histological results) Without exception they were diagnosed correctly as far as their sex was concerned.

Included in the group of 52 male pseudohermaphrodites there were 20 cases of so-called testicular feminization which corresponds to the ratio found by Money (United States) Hæmatologically all of them showed undoubted male nuclear sex. Fi e investigated elsewhere by means of skin biopsy oral smear or blood smears gave the same result. One or more cases of true hermaphroditism may be hidden among the 25 hermaphrodites with male nuclear sex which were only diagnosed clinically In 12 control examinations conducted elsewhere the negativity of the sex chromatin was confirmed (in one case however the skin biopsy diagnosed by an experienced investigator at first led by mistake to a chromatin positive diagnosis and only after repeating the skin biopsy and making a blood and oral smear examination was the nuclear sex demonstrated to be male)

It must be admitted that there are some cases of male pseudohermaphroditism, we saw 4, not by the way examples of typical testicular feminization where hæmatological recognization was complicated by the presence of a number of unusually large small clubs" as well as other nuclear appendages It is possible that further detailed investigation of these might demonstrate chromosomal anomalies and create the necessity for more clinical groups.

It should be mentioned that in the uppermost group on the right side of Table II, approximately *half of the children under* the age of two listed in this group were registered wrongly! In addition, among these 55 cases there were 6 with the absolutely male type of external genitalia, which has been described only five times previously Only 12 cases of non adrenal female

TABLE 11

HÆMATOLOGICAL "NUCLEAR SEXING" IN CASES OF PSEUDOHERMAPHRODITISM

(Case history and sex unknown prior to the investigations!)

Diagnosis	Number of Cases	Hæmatological Findings	Percentage of Agreement	Diagnosis	Number of Cases	Hæmatological Findings	Percentage of Agreement
MALE PSEUDOHERMAPHRODITISM Diagnosis confirmed	5	5 × ♂ (7 cases investigated by other examiners—skin biopsy oral or vaginal smear or hæmatological investigations—showed the same result)	00	CONGENITAL ADRENAL [illegible] Diagnosis confirmed	55	55 × ♀ (average number of drumsticks 4/500) (cases investigated by other examiners—skin biopsy oral smear or hæmatological—same result)	00
				Clinically suspected cases Female [illegible] post natal adrenal [illegible] [illegible] tumour	5	5 × [illegible] (average number of drumsticks 8/500) (oral smear same result)	00
Final diagnosis on clinical basis only	5	5 × ♂ (cases investigated by other examiners—skin biopsy oral smear or hæmatological—same result)	00	FEMALE PSEUDOHERMAPHRODITISM WITHOUT ADRENAL DISORDER Diagnosis [illegible] by means of biopsy or autopsy	[illegible]	5 [illegible] 4 and 7 drumsticks /500	00
				Final diagnosis on clinical basis only	[illegible]	5 (and drumsticks /500)	00

77

7

pseudohermaphroditism have been observed according to Wilkins *et al.* (1955) and Lenz (1957) states that only 13 have been published The three cases on the right side of Table II without adrenal disorder could include a case of true hermaphroditism.

Table III shows the grouping in gonadodysgenesis. The sexes are distributed approximately in the same ratio of one with

TABLE III
HÆMATOLOGICAL "NUCLEAR SEXING" IN CASES OF GONADAL DYSGENESIS
(Case history and sex unknown prior to the investigations!)

	Number of Cases	Hematological Findings ♂	Hematological Findings ♀
Diagnosis established (Operatn and histologically)	22	6 (cases investigated by other examiners—skin biopsy or hematological investigations—showed the same result)	6 (average number of drumsticks 5/500 (3 cases investigated by other examiners—skin biopsy or hematological investigations—showed the same result
2. Diagnosed clinically (Hormonal studies made and laparotomy but withou histological investigation)	53	44 (4 cases investigated by other examiners—oral smear or hema ological—same resul)	9 average number of drumsticks 6/500) (5 cases investigated by other examiners—oral smear or hematological—same result)
3. All other suspected cases	8	6	
	83	66 ♂	7

female to four or five with male nuclear sex as Nelson found in the United States in 1956 in a rather larger series. These two separate investigations accordingly support one another

We also noted several times (as in the cases of male pseudohermaphroditism in the limited sense) the large small clubs" in cases of gonadal dysgenesis with male nuclear sex (also called perfect male pseudohermaphroditism) These occasionally complicated the diagnosis. As with Prader we were also unable to make a diagnosis of the nuclear sex in some rare cases. In the uppermost group of the gonadal dysgenesis on the right side of Table III we encountered one clear misdiagnosis by an experienced colleague from the skin biopsy (93 male nuclei") on

account of the blood smear result—30 and on repetition 61 drumsticks per 500 neutrophils—the biopsy was repeated and now found to be typically female

As the different problems with which Klinefelter's syndrome confronts us today especially those arising during the hematological diagnosis of the nuclear sex, have been discussed already by the most competent investigators I shall refer therefore only briefly to our investigations Besides the cases easily diagnosed hematologically even at the first glance chromatin positive (first group in Table IV) there exists others only recognizable as chromatin positive with difficulty (second group) In these n may have to count several thousand leucocytes while isolat d cases in a third group could not be diagnosed hematologically at all From this one is tempted to assume a clear superiority of other methods of nuclear sexing particularly as in some of these cases a clear chromatin negative misdiagnosis had been mad by other experienced hematologists—but this is too simple a n ception as the fourth group with its great discrepancies n th findings shows.

Groups 1 to 4 represent the true Klinefelter's syndrome of Nelson and in group 5 are listed the cases of false Klinefelter's syndrome of this author Undoubtedly refinement of the methods of cytological examination will yield further information

Even though it is true that the direction of the sexual desire does not by any means depend solely and directly upon the nuclear sex and the nature of the gonads, it is my opinion that detailed and refined investigations are also indicated in those states of mental intersexuality whose origin, whether pur ly genetic or purely psychological is still undecided. In 1955/56 Professor Bleuler from Zurich and I relying upon the outlines of Barr and Hobbs, investigated hematologically 28 constitutional homosexuals and ten transvestites including in the latter group for the first time eight women In every case we found the nuclear sex to be the same as the social and genital sex

Please let me discuss two more points Besides the differences in the frequency of drumsticks in healthy females of the same ag which are often very noticeable and remain constant, there exists also an age-dependence in the frequency of drumsticks in healthy persons This was first described by v Harnack and Strietzel myself and others On the left side of Table V the average counts of drumsticks per 500 leucocytes came from a series of healthy and on the right side from sexual abnormal individuals investigated in our clinic

It is evident that the number of sex-characteristic nuclear

TABLE IV

HÆMATOLOGICAL "NUCLEAR SEXING" IN FIFTY CASES BELIEVED TO SHOW KLINEFELTER'S SYNDROME

(Case history and sex unknown prior to the investigations!)

Clinically	Our Hæmatological Result	Number of Cases	Remarks to the Hæmatological Findings	Testicular Biopsy made elsewhere. Nuclear Sex Diagnosis from Oral Smear and/or Leydig Cells
1. Klinefelter syndrome	Typical ♀	9	Average number of drumsticks 3–6/500 No peculiarities	Testicular biopsy taken in 5 cases: all typical Nuclear sex determined in 7 cases (oral smear and/or Leydig cells): all typical ♀
2. Klinefelter syndrome	Doubtfully ♀" or "likely ♀"		Atypical few drumsticks" (always less than 6/500)—averaging 86/500 Suspicious signs of chromatin positively regularly present (drumstick-resembling, but small appendages, also intranuclear chromatin densities) Numbers of small clubs" as well as sessile nodules varying and uncharacteristic	Testicular biopsy in 6 cases: all typical Nuclear sex determined in 4 cases (oral smear and/or Leydig cells): all typical ♀

3. Klinefelter syndrome	"No final diagnosis possible	3	Examination by another experienced hematologist in one of these cases no final diagnosis possible	Nuclear sex determined in cases (oral smear) typical ♀
4. Klinefelter syndrome suspected	"Probably ♀" "Probably ♀" "Probably ♂" "No diagnosis possible "No diagnosis possible "Probably ♂"	(6)		Oral smear atypical ♀" Oral smear "no diagnosis possible Oral smear ♂ Oral smear ♂ Oral smear ♂ Oral smear typical ♀"
5. Klinefelter syndrome suspected	Typical ♂		—	Testicular biopsy in 3 cases non-typical Nuclear sex determined in 8 cases (oral smear and/or Leydig cells) all typical ♂
6. Puberty gynecomastia, pituitary hypogonadism, hypogonadism post-parotitis—other cases referred rth misdiagnoses	Typical ♂	9	—	Nuclear sex determined in 6 cases (oral smear) all typical ♂

appendages in healthy as well as in sexually pathological girls and women declines from infancy through school age to adult life. I cannot give you any explanation for this phenomenon of the age-dependence, nor for the frequency-differences in drumstick counts in individuals of the same age One is tempted to consider differences in the intensity of the metabolism as have been

TABLE V

RELATIONSHIP BETWEEN THE AGE AND AVERAGE NUMBER OF DRUMSTICKS"

A erage Number of "Drumsticks /500 Neutrophil Leucocytes in Cases of Healthy ♀ Individuals		Average Number of Drumsticks /500 Neutrophil Leucocytes in Cases of Sexual-abnormal ♀ Individuals	
I 49 cases of premature and new-babies	[illegible]7·5	I 18 cases of babies	9·4
(Our group publ. 957)		(Our group, publ 957)	
Premature [illegible]8		I 4 cases of babies	8·9
Newborn babies [illegible]3·7		(Our group 957 not publ.	
I 00 cases of children of all ages	3·[illegible]	In cases of infan	7·4
(Kosenow and Scupin publ.	10	(Our group 957)	
1956)	7·5	In 3 cases of children of school age	7
In cases of children of school age	-4	(Our group 957)	
(Our group publ. 955)		I 29 cases of adults	9·4
I cases of individuals of all age groups	3·8	(Our group publ. 957)	
(Davidson and Smith publ.	t	I 63 cases of adults	9·5
954)	3·9	(Our group 957 not publ	

suggested by Sachs and Danon in connection with their skin-findings. This supposition becomes especially obvious, if one begins to doubt if the so-called sex-chromatin is a primary sex phenomenon It has been said that the sex-chromatin in the skin and mucous membranes is not age dependent (Lennox, 1956 Dixon and Torr 1956 Lupatkin and Prader 1956) but I am unable to say whether these investigations have been sufficiently extensive. It may well be that some of you know the answers to these questions.

In closing ladies and gentlemen I would like to mention briefly the confusion of the medical terminology which exists in the literature. A multiplicity of terms such as primary "chromosomal genetic "zygotic" "true" as well as "actual 'real (and so on) sex are used. Furthermore the different usage of desig nations such as testicular dysgenesis applied both to cases of gona dal dysgenesis with male nuclear sex and to Klinefelter s syndrome is disturbing Finally individuals with Klinefelter s syndrome with positive sex-chromatin and thus a discrepancy between gonadal

and nuclear sex have been called either "female" pseudohermaphrodites (in spite of the presence of testes) or by others, "male pseudohermaphrodites" (in spite of the presence of male external and internal genitals).

A clarification of nomenclature is urgently required before this confusion becomes even greater.

I propose, therefore, particularly in view of the fact that both Dr. Lennox and Dr. Barr have in the last year proposed terminological modifications—that this convention should agree to a terminology which can be used internationally, at least for the time being.

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FREQUENCY OF KLINEFELTER'S SYNDROME AND THE RELATIONSHIPS OF CHROMATIN POSITIVE AND CHROMATIN NEGATIVE CASES

BERNARD LENNOX M.D Ph.D M.R.C.P
M. A. FERGUSON-SMITH M.B Ch.B.,
W S MACK, F.R.C.S (Ed.) and
J S S STEWART M.B., Ch.B
Departments of Pathology and Urology Western Infirmary
University of Glasgow

I MUST apologize for presenting material some of which has already appeared in print (Ferguson-Smith, Lennox, Mack, and Stewart, 1957) especially as we have some more recent findings to be given by Dr Stewart, but our earlier more mundane observations perhaps warrant some discussion at this meeting

First of all, a suggestion on nomenclature. We need an alternative to Klinefelter's syndrome and "seminal tubular dysgenesis and the like are unsatisfactory Dr Ferguson-Smith has proposed congenital micro-orchidism' It seems to me crisp and accurate, and well worth consideration

STATISTICS

We have a fairly large collection of cases now (24 chromatin positive in all for instance) but what I have to say is chiefly concerned with a series collected as a result of a deliberate search through the material of Mr Mack's male infertility clinic. Believing it to be unlikely that we would find any cases with sperm counts over 1 000,000, we confined our attention to the 126 cases with oligospermia below that figure or with complete azoospermia. *Eighty-six of these responded to a request to return* for re-examination An oral mucosal smear showed 10 to be chromatin positive These were all included in a group of 27 cases regarded on purely clinical grounds as examples of Klinefelter's syndrome Detailed re-assessment of these 27 cases is in progress, and none have yet had to be rejected as a result.

Among the original 126 cases 69 had already had testicular

biopsies on purely histological grounds 14 of these were called Klinefelter's syndrome 8 being chromatin negative and 6 chromatin positive.

From our statistics three points seem to emerge (*a*) Chromatin negative cases seem to be commoner than chromatin positive (*b*) This is a condition of real practical importance in a male sterility clinic if the large sample we have examined is representative 30 per cent of all our patients with azospermia or gross oligospermia are cases of Klinefelter's syndrome, and one in thirty of all patients attending the clinic with significant deficiency of the sperm count ($<$40 000 000 per ml.) is a genetic female (*c*) We can make a first approximation towards an estimate of absolute frequency A sterility clinic draws most of its patients from a limited age group In the age decade of greatest frequency 28–37 years, there are 4 chromatin positive and 5 chromatin negative cases with Glasgow addresses The million inhabitants of Glasgow include some 71 000 of either sex in the relevant decade. This gives a crude incidence of 1/18 000 for positive and 1/14,000 for chromatin negative cases. Even in this decade we do not pretend to have collected all the cases in Glasgow and making all allowances for random error we think the real figures must be substantially higher than those given—probably well over 1/10 000 for each variety This must be, for instance, a much commoner disease than hæmophilia. If then we can accept as true the view that there is a top limit of 1/50 000 (at most) for mutations at one locus, it is certain that neither form of the disease can be the result of single mutation at a single locus—unless, that is, one mutation can produce several cases.

HISTOLOGY

Dr Siebenmann is to describe the detailed histology of the testis in chromatin positive cases, and I am only concerned here to point out the differences between the two forms of the disease. Nelson (1956) was first to point out the existence of such differences (we only recently became aware of this paper) but Grumbach, Blanc, and Engle (1957) have doubted the complete distinctness of the two forms. In the chromatin negative cases the Leydig-cell hyperplasia is diffuse and even the cells normal. The tubules are well preserved, *less than 50 per cent being totally* hyalinized. Most are lined by Sertoli cells. Total lack of all spermatogenic elements is exceptional, and often a substantial proportion of tubules show at least a few spermatogonia. In the chromatin positive cases, the Leydig cells are clumped into solid, almost tumour like masses and the cells show considerable

nuclear irregularity The tubules are irregular in size and fewer in number over 70 per cent are hyalinized. Elastic tissue is grossly deficient. Curiously anomalies such as the growth of Leydig cells within tubules appear occasionally Spermatogenic elements are present in far fewer cases and in only a few tubules when present at all. (Note, however our 4 cases with some spermatozoa in Table I also a case to be published by one of

Table I
INCIDENCE OF KLINEFELTER'S SYNDROME IN A MALE INFERTILITY CLINIC

	Number of Cases	Oral Smear Obtained	Nuclear Sex Female	Nuclear Sex Male
Total patients attending 1955–56	83	—	—	—
Sperm counts available	738	—	—	—
Extreme oligospermia (< 10^6/ml)	76	49	4	45
Azoospermia	50	37	7	30
Clinical diagnosis of Klinefelter's syndrome	17	7	1	6
Testicular biopsies available	69	—	—	—
Histological diagnosis of Klinefelter's syndrome	4	—	6	8

us—Ferguson-Smith and Munro 1958—with unusually well developed spermatogenesis in one tubule.) We have never found any difficulty in predicting the nuclear sex from a rapid survey of the testicular biopsy with a low power lens.

These findings at least made it quite clear that the two forms of the syndrome are genuinely distinct and that the difference of nuclear sex is not a purely incidental finding When we first made this observation, we inclined to the view—which is I think a fairly general one—that whereas the chromatin positive cases form an outstandingly interesting and unitary group the chromatin negative cases would be found to be a loose assortment of various forms of testicular degeneration of less immediate interest. As Dr Stewart will tell you we have since had reason to alter that view Finally while the chromatin negative testis is clearly a testis and has never been anything else the chromatin positive testis is so grossly abnormal that we wonder if it may not at some stage have been an *ovary and I for one suspect that some part* of its testicular differentiation may be post natal, under the influence of its scrotal position.

SUMMARY

1 Klinefelter's syndrome is responsible for a substantial fraction of cases of male sterility in Glasgow

2 Chromatin negative cases are commoner than chromatin positive in our material, and neither form is likely to have a frequency of less than 1/10 000 men in Glasgow

3 Histologically the two forms are quite distinct

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DISCUSSION

This paper was discussed with papers 16 and 17 See p 127

16

GONADAL HISTOLOGY AND NUCLEAR MORPHOLOGY IN KLINEFELTER'S SYNDROME

R. E. SIEBENMANN M.D
Institut of Pathology University of Zurich

DURING the last five years we have examined 16 "testicular" biopsies and 4 complete autopsies of apparent males whose nuclear sex—by subsequent or simultaneous determination—proved to be female. This material was found by re-examination of all cases which histologically showed a tubular fibrosis with persistent or increased Leydig cells or a so-called germinal aplasia and of the testicular biopsies from those patients in whom the clinician suspected Klinefelter's syndrome

The clinical symptoms of these 20 cases with female nuclear sex may be summarized briefly as follows Their age ranged from 14 to 72 years. Moderate androgen deficiency was present in 15 cases and a gynaecomastia in 15 cases. F.S.H. excretion was significantly raised (over 96 m.u.) in 11 of 14 studied cases It was normal in an 18-year-old patient, in a 14 year-old boy in whom it was found elevated two years later and in a 31 year-old patient whose urinary estrogen-excretion was raised.

Nuclear sex was determined in the Leydig cells. The percentage of chromatin positive nuclei varied between 41 and 59 per cent In the majority of cases nuclear sex was also determined in the oral mucosa smear by Dr Prader and/or in blood smears by Prof Wiedemann

Only 1 of the 20 cases showed a genital malformation, with hypoplasia and slight hypospadia glandis and incompletely descended testicles. There was no trace of a female genital structure in the 4 autopsy cases. The testicles were always very small In the living patients their length never exceeded 2.5 cm. their volume ranged between 2 and 4 c.c. The combined weight in the autopsy cases ranged from 2·7 to 4·0 g

The histological examination of many sections of the often bilateral biopsies and whole gonads in the autopsy cases revealed the following common characteristic features

1 There is an irregular arrangement and distribution of the

tubules and tubular scars, some of them lying close together others being separated not only by accumulations of Leydig cells but also by a loose connective tissue

2 There is always an association of partially or completely sclerosed tubules and tubules with no sclerosis at all even in older patients (Figs. 1 and 2)

3. The non-sclerosed tubules vary greatly in size (Fig 3) Some of them attain almost normal adult diameters of over 100 micra others are very small with diameters found in the pre puberal testis. As this reduction in size is not associated with thickening of the tubular wall it cannot be the result of scarring Th tubules show a small lumen or no lumen at all. They are mostly lined by Sertoli cells only which are usually well preserved if there is no sclerosis of the wall. These tubules closely resemble those found in so-called germinal aplasia of Del Castillo With increasing tubular sclerosis the Sertoli cells show marked nuclear and cytoplasmic degeneration

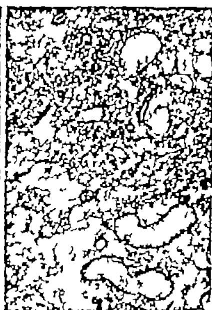
When germ cells occur they are found in a few single tubules (usually of the larger adult size) always surrounded by tubules with Sertoli cells only Germ cells were found in 6 of our 20 cases spermatogonia were present in a 1, and a 19-year-old boy spermatocytes were found in an 18 and a 48-year-old patient and spermatogenesis was complete in a 14 year-old boy at the beginning of puberty and in a 31-year-old man (Fig 4) The latter however suffered from azoospermia.

4. A delicate argentophil basement membrane is always present as long as the tubule is lined by Sertoli or germ cells. On the other hand elastic membranes, *which normally appear in the tubular* wall at puberty are only present in some of the larger tubules and many of the smaller tubules are devoid of elastic tissue even in older patients (Figs 5 6, and 7)

5 Tubular sclerosis is due to a deposition of hyaline or finely fibrillar material outside the intact basement membrane but inside the elastic membranes when they are present It is sometimes found only in a sector of the tubule. After complete scarring of the tubule the basement membrane disappears whereas the elastic membranes once formed persist. The hyaline tubular scars vary greatly in size and there are always some small scars completely devoid of elastic tissue remnants. This indicates that these tubules either have undergone sclerosis before puberty or have never matured (De la Balze *et al.*, 1954)

The typical tubular sclerosis of Klinefelter' syndrome with female nuclear sex begins only with puberty (probably before any increase in Leydig cells) but it seems to be preceded by marked retardation or regression of germinal epithelium development (*Siebenm.. and Wyder* (1958, 88, 607)







3



4

6 There is a clear-cut progression of tubular sclerosis and a probably relative increase of Leydig cells with increasing age. The result is either a nodular arrangement of Leydig cells with much loose connective tissue thick walled vessels, and tubular scars or large fields of interstitial cells with only sparse connective tissue and very few sclerosed tubules The Leydig cells are usually well preserved in the younger but exhibit marked degenerative nuclear polymorphy and shrinking of the cytoplasm in the older patients A dense reticular network between them is collagenized in the older patients and the result is an often marked fibrous scarring in the clumps and fields of Leydig cells. Many sections through the whole gonads in the 4 autopsy cases were examined and no ovarian tissue was found. The rete tubules were hyperplastic, and lined by a high columnar epithelium. In all 4 cases abundant typical perineural hilus cells were found

These are the essential features found in our 20 chromatin positive cases Most of them have been reported by other authors too (Grumbach *et al.*, 1957 Jackson *et al* 1956 Nelson, 1956 Witschi *et al.*, 1957) but in contrast to their reports until now we *have never found them in chromatin negative cases.*

In the same five year period we found only two biopsy specimens from chromosomal males with hypogonadism increased F.S.H excretion (over 96 m u.) and gynaecomastia (one having also azoospermia) but the biopsies showed nothing but a disturbance of spermatogenesis without tubular sclerosis or increased Leydig cells.

Only one patient was found who may be considered as an

FIGS. –8 *Testicular dysgenesis in apparent males with female nuclear sex.*

FIG. Low power aspect showing irregular arrangement and varying size of patent tubules and tubular scars separated by Leydig cells and loose connective tissue 8-year-old boy without gynaecomastia and normal F.S.H. excretion. Biopsy 758/57 an Gieson, ×40.

FIG. 2. Low power aspect of advanced changes. Most of the tubules are completely or incompletely sclerosed, but there are still some non-sclerosed tubules lined by Sertoli cells only or showing incomplete spermatogenesis. The number of Leydig cells has increased. 48-year-old man with gynaecomastia and increased F.S.H. excretion. Biopsy 5073/54, Iron-Hematox. Eosin, ×40.

FIG. 3. Detail of Fig Non-sclerosed tubules of varying size lined by immature and mature Sertoli cells, reminiscent of the so-called germinal aplasia Very small tubular scars. 8-year-old boy Biopsy 758/57 an Gieson, × 00

FIG. 4. Complete spermatogenesis with several spermatocytes in single tubule. 4-year-old boy with gynaecomastia; F.S.H. excretion, normal at time of biopsy was elevated years later Female nuclear sex was found in Leydig cells, oral mucosa, and blood smear Biopsy 498/54, an Gieson, 450.



5



6



7



8

example of "Klinefelter's syndrome in a chromosomal male He had a history of operated cryptorchism, no hypogonadism, no gynæcomastia, but a significant increase of F.S.H excretion and a *varying degree of tubular sclerosis with increase of Leydig cells.* However there were no small, immature non-sclerosed tubules and all the tubular scars showed elastic tissue remnants.

This *is an example of what we consider to be a primary testicular* atrophy (i.e. not secondary to hypopituitarism) We found several other cases of atrophy without obvious cause and no clinical signs other than oligo- or azoo-spermia. Histologically these atrophies are characterized by tubular sclerosis and persistent or increased Leydig cells too, but we always found it possible to distinguish them from the peculiar changes in patients with female nuclear sex.

This was especially true in seven cases of dystrophia myotonica with testicular atrophy which, in contrast to the one patient of Grumbach *et al* (1957) all had male nuclear sex. They had moderate hypogonadism but no gynæcomastia. F.S.H excretion was moderately elevated in two cases studied (66 and 96 m.u respectively)

Three biopsy and four autopsy specimens show that tubular scars and incompletely sclerosed tubules are regularly distributed the latter always with some germ cells in the more or less degenerated germinal epithelium. Immature tubules without fibrosis, or tubules lined by preserved Sertoli cells only are never found *The tubular scars are almost all of the same size and always sur*rounded by elastic tissue remnants. The Leydig cells may be increased as small clumps, but they also form larger nodules (Fig 8)

These changes suggest a primary testicular atrophy In two

FIG. 5. Tubular sclerosis Hyaline material is laid down outside the inner basement membrane often only in one sector The basement membrane disappears after complete sclerosis. Dense network of reticulin fibres between the Leydig cells. 48-year-old man. Biopsy 5073/52 Foot, × 00.

FIG. 6. Tubular sclerosis Elastic membranes in the wall of patent larger tubules. In the lower half an immature small tubule without lumen and without elastic tissue in the wall. Most of the tubular scars are devoid of elastic tissue remnants. 48-year-old man. Biopsy 5073/52 Resorcin-Fuchsin, × 00.

FIG. 7 Irregular distribution and varying size of tubular scars, some of them showing scanty elastic tissue remnants, others none at all. Clumps of Leydig cells. 54-year-old man. Autopsy 498/55. Resorcin-Fuchsin, × 00.

FIG. 8. Testicular atrophy in patient with dystrophia myotonica and male nuclear sex.

Tubular sclerosis with nodule of persistent Leydig cells. The tubular scars are almost all of the same size and all surrounded by elastic tissue remnants. 65-year-old man Autopsy 740/57 Resorcin-Fuchsin, × 00.

autopsy cases however Leydig cells were present only in a few scattered nodules. The impression that in these cases a certain degree of hypopituitarism was responsible for the testicular atrophy was supported by the finding of a diffuse atrophy and fibrosis, and a cystic scar respectively in the adenohypophysis. This is in agreement with the clinical observations and review of literature of Mertens and Nowakowski (1954)

CONCLUSION

Our material reveals a characteristic histological picture in the gonads of phenotypical males with female nuclear sex and the more or less complete clinical symptoms of Klinefelter's syndrome. It differs from primary testicular atrophy in chromosomal males, even if they should clinically exhibit symptoms of Klinefelter's syndrome I agree therefore with Lennox (Ferguson-Smith *et al.*, 1957) who found it possible to distinguish histologically between male and female cases. Still I do not think they differ in the behaviour of the Leydig cells, but do in the changes in the tubules. The tubular changes in the chromatin positive cases are indeed suggestive of a dysgenetic origin. They may indicate a failure of the cortical anlage with inversion of gonadal sex in genetic females or a medullary (male) gonadal development in individuals with a female like genetic constitution (shown in the female like nuclear morphology) The subsequent development of this medulla is, however very defective as far as the tubules are concerned, whereas the interstitial portion, i.e the Leydig cells, are morphologically and functionally much less impaired by the discordant genetic surrounding

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DISCUSSION

This paper was discussed with papers 15 and 17 See page 127

NATURE OF THE GENETIC DEFECT IN KLINEFELTER'S SYNDROME: EVIDENCE FROM FAMILY AND BLOOD GROUP STUDIES

J. S. S. STEWART, M.B., Ch.B., MARIAN M. IZATT, B.Sc.,
M. A. FERGUSON-SMITH, M.B., Ch.B.,
B. LENNOX, M.D., Ph.D., M.R.C.P. and
W. S. MACK, F.R.C.S. (Ed.)

Departments of Urology and Pathology, Western Infirmary,
University of Glasgow
and the West of Scotland Regional Blood Transfusion Service

I should like to describe some of the interesting findings discovered in a study of our cases of Klinefelter's syndrome at Glasgow. Since time is short I trust that you will excuse a somewhat dogmatic presentation of these findings.

CHROMATIN NEGATIVE CASES

Firstly let us consider how genetic transmission of the defect might occur. Chromatin negative cases of Klinefelter's syndrome are apparently feminized males. If the defect were dominant the father could not have transmitted it for he would have been affected and therefore sterile. A feminized female might, however, be normal, at least in respect of fertility, and it is possible that the mother of a case might transmit such a dominant defect. If so we should look among her brothers for additional cases. The siblings of the parents of the 16 chromatin negative cases include four groups—paternal uncles, paternal aunts, maternal uncles and maternal aunts (Table I). Each group can be further divided into those who died young (D) before the age at which they might be expected to marry, those who remained celibate (C), those who were married but childless and may be assumed to be sterile (S), and those who were married with children and are therefore apparently fertile (F). For each group the ratio of *childless to fertile adults* $\left(\frac{C+S}{F}\right)$ *can be calculated and the total* can be obtained by adding together all four sub-divisions. It is

TABLE I

KLINEFELTER'S SYNDROME—PARENTAL SIBLINGS OF SIXTEEN CHROMATIN NEGATIVE CASES

	Died Young (D)	Celibate (C)	Sterile (S)	Fertile (F)	Ratio of Childless to Fertile Adults (C + S)/F
Paternal uncles	2	5	2	12	7/12
Paternal aunts	1	2	5	6	7/ 6
Maternal uncles		9	4	5	13/15
Maternal aunts	1		3	14	4/14

(C + S)/F for maternal uncles = 2·5 } $\chi^2 = 5·802$
(C + S)/F for others = 0·62 } $p <$ 0·02

clear that there is an excess of childless maternal uncles and this excess is statistically significant ($\chi^2 = 5·802$ $p < 0·02$) It should be noted that the childlessness is mainly due to the large number of celibate maternal uncles. Eunuchoidism, a feature of Klinefelter's syndrome, may have prevented marriage and it seems reasonable to add together those celibate and those sterile in calculating childlessness

CHROMATIN POSITIVE CASES

The chromatin positive cases are apparently genetic females who have been masculinized The mother could not transmit such a dominant defect, but a masculinized male might well be fertile and the defect could be transmitted by the father If so additional cases might occur among the father's brothers An analysis of parents siblings of 9 chromatin positive cases (Table II) can be used to estimate childlessness as in the chromatin negative cases. In the chromatin positive cases an excess, probably significant, of childless paternal uncles is present ($\chi^2 = 4·532$ $p < 0·05$) Since sex reversal occurs in chromatin positive cases we would expect to find a disturbance of the sex ratio if some of these uncles are chromatin positive cases and in fact a disturbance probably significant, does occur ($\chi^2 = 4·064$ $p < 0·05$)

One-third of our cases show evidence of inheritance. A normal sex ratio is found among siblings of chromatin positive cases but this could be due to the smaller family size in this generation coupled with a high "mutation rate" producing a large proportion of sporadic cases.

TABLE II
KLINEFELTER'S SYNDROME—PARENTAL SIBLINGS OF NINE CHROMATIN POSITIVE CASES

	Died Young (D)	Celibate (C)	Sterile (S)	Fertile (F)	Ratio of Childless to Fertile Adults (C + S)/F
Paternal uncles	0		4		5/
Paternal aunts			4	8	6/8
Maternal uncles		4			5/
Maternal aunts				4	4/ 4

(C + S)/F for paternal uncles = 5 } χ^2 = 4·550
(C + S)/F for others = 3 34 } $p <$ ·05
Discovered total (D + C + S + F) for paternal uncles = 37
Discovered total (D + C + S + F) for paternal aunts = 5
Discovered sex ratio = 37 5 } χ^2 = 4·064
But expected sex ratio = 27 25 } p ·05
(05 males to 00 females at birth)

IMPLICATIONS OF FAMILY STUDIES

These findings indicate that

1 Chromatin positive and chromatin negative Klinefelter's syndrome are similar diseases.

2 Both are inherited

3. The mechanism of inheritance is similar

4. The chromatin positive cases are, as nuclear sexing suggests, genetic females

5. The mechanism of inheritance is that of a sex controlled dominant, the defect in chromatin positive cases being transmissable by the father and in chromatin negative cases by the mother

BLOOD GROUP STUDIES

The possibility of heteroplo dy or chromosome mutation (i e chromosomal aberration) as the cause of Klinefelter's syndrome was considered. The blood group antigens were examined using a master dilution titration technique to detect triplication, deletion or other abnormalities. In two cases, one chromatin positive and one chromatin negative, unusual Rhesus antigens were discovered In each case apparently heterozygous (since agglutination occurred with Anti E and with Anti-e sera) titration gave a result intermediate between that expected of a homozygote (EE) and of a heterozygote (Ee) but nearer to the former The

strength of Rhesus antigens does vary and these results could be due to chance alone. Nevertheless the possibility that the findings are due to an abnormal genetic environment or to triplication must be further explored.*

EXAMINATION OF RELATIVES

Extensive examination of relatives has not so far been possible but some information is of interest.

1. A chromatin negative case has a brother who is grossly eunuchoid. This brother has been examined and his testes are normal in size (approximately 5 cm. each in longest diameter).
2. Another chromatin negative case has a maternal uncle who may be schizophrenic.
3. A chromatin positive case had a paternal uncle, now dead, who is said to have had small testes.

GENETIC CONSIDERATIONS IN KLINEFELTER'S SYNDROME

A genetic mechanism operating in Klinefelter's syndrome must be satisfactory for chromatin positive and chromatin negative cases and must also account for the following reported facts.

1. The occurrence in a sibship of
   (*a*) Chromatin positive and chromatin negative cases of the syndrome (Grumbach *et al.* 1957).
   (*b*) Klinefelter's syndrome and Turner's syndrome (Bassöe, 1956).
2. The association of Klinefelter's syndrome with
   (*a*) Dystrophia myotonica (Grumbach *et al.* 1957).
   (*b*) Laurence Moon-Biedl syndrome (Francke, 1930).
   (*c*) Osseous abnormalities of the type apparently sometimes seen in Turner's syndrome (Sohval and Soffer 1953).
3. The association of Turner's syndrome with dystrophia myotonica (Nadler *et al.*, 1950).

NATURE OF GENETIC DEFECT

The reported facts could be explained by a chromosomal aberration involving the autosomal masculinizing (M) loci with triplication in the chromatin positive genetic female (XX) cases and deletion in the chromatin negative genetic male (XY) cases. Autosomal translocation is one type of aberration which could explain the facts.

* Further investigations, not yet completed, have shown that the account of the Rhesus anomaly presented to the Symposium was an oversimplification and that the association with Klinefelter's syndrome may prove to have been fortuitous.

1 Chromatin positive cases would be masculinized females (MMMXX)

2 Chromatin negative cases would be feminized males (MXY)

3 A reciprocal translocation in a grandparent with a balanced translocation in either parent could by segregation, give chromatin positive and negative cases in a sibship

4 Associations with other syndromes could be due to involvement of the appropriate loci in the translocation.

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DISCUSSION on paper 15 16 and 17

BARR Before we go any farther I wonder whether it would not be wise to see whether we are in agreement as to the criteria of Klinefelter's syndrome. If we are not talking about exactly the same thing then the proportions of those that are male and those that are female as far as the nuclei are concerned don t mean very much It seems to me that the only real requirements are the small testes and the fibrosis and hyalinization of the seminiferous tubules, and that other features may or may not be present. A debatable point may be the gonadotropin level we have had two amongst our patients who have normal levels, so it is not necessarily elevated—others may have different views on that. I wonder whether there is any agreement on that general definition. The criteria have changed several times since the original papers of Klinefelter

STEWART To confuse the picture still further one of our chromatin negative cases has a eunuchoid brother with normal size testicles. He has female distribution of pubic hair and he shaves once a week. His span is 6 ft. 2 in. and his height 5 ft. 8 in. Would you also fit him into the syndrome despite the size of his testicles?

EMERY A short while ago I was shown two testes by a pathologist—the testes were identical with what is shown in Klinefelter s syndrome yet the pathologist was quite convinced they were due to mumps.

HARRIS In Stewart s family stud es of the chromatin positive cases there was an excess of males in the paternal sibship. The

underlying idea is, I take it that among those males there are some genetical females. Have you actually looked to see whether they are actually chromatin positive or negative?

STEWART We are starting to do that, but it is going to be very difficult indeed to follow them up One chromatin positive case did say that he had a paternal uncle who had very small testicles Unfortunately that uncle is now dead. I hope to see some of the *mothers of these cases* and enquire about abortions and so on which may explain to some extent why the discrepancy does not appear in the sibs of cases. In one family I hope to examine in particular among the paternal uncles and aunts there were eighteen males and two females.

HARRIS But this is just in one family?

STEWART Yes.

HARRIS It seems to me there is a great danger if the thing is studied in just a few families because in one family there may be intrafamilial causes to make them celibate or something of that sort. The other question I wanted to ask was that it appears that the frequency of chromatin positive cases is beginning to approach a range where even in random searching people looking at blood smears, ought to be picking them up by chance. I was wondering if that had happened

DAVIDSON We have actually picked up some by chance in the scanning of routine blood films One was found by chance from the asthma clinic.

BISHOP Could I just ask Dr Stewart a question—these enquiries that you made about paternal aunts and uncles—did you in actual fact succeed in interviewing all these uncles?

STEWART No

BISHOP I mean most people would not know the size of the testicles of their uncles!

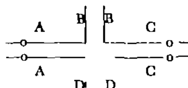
STEWART All I can say sir is that this particular patient volunteered the information. How he obtained it I do not know

FORD Dr Stewart, am I to understand that in your explanation of the inheritance of this condition that you postulate a three to one segregation of the four translocation chromosomes in the association—postulated association—at the first metaphase of meiosis to account for it?

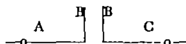
STEWART No in fact I was hoping you might be able to give me some guidance as to what happens to a balanced translocation under these circumstances. Would you in fact get one quarter normal, one quarter balanced translocation and one half unbalanced translocation?

FORD We have quite a lot of evidence in mice from some

twelve different translocation lines and I might say that this evidence fits in with a lot of experience of translocations in other animals, invertebrates and in plants. The situation can be represented AB BC, CD DA, and let the centromere be indicated by small circles.



Now in approximately 50 per cent of cases one gets a disjunction, so that two adjacent chromosomes go to one pole and a segment is duplicated—B in this instance—and a segment is deficient (D)



These 50 per cent of cases give unbalanced sperm or ova. They are viable as can be demonstrated by an ingenious experiment of Snell's If fertilized by a balanced gamete of either type (AB CD or AD CB) they give an unbalanced zygote, but if fertilized by a complementary unbalanced gamete (e.g AB BC *plus* CD DA)—which can only occur in the mating of one animal heterozygous for the translocation by another animal heterozygous for the translocation—then you can get a viable, balanced zygote But there is just no evidence whatever for a viable gamete coming from a three to one disjunction of the chromosomes, which does occur though rarely and I thought that was essential for your argument.

STEWART No I was postulating simply that sometimes you got alternate disjunction. On the diagram there is a chromosome with A and D and also a chromosome with B and C on it you get the opposite pairs.

FORD You do I described the 50 per cent of unbalanced types—the other 50 per cent occur when AB and CD go to one pole AD and CB to the other They give you a full set of four chromosome segments and the gamete is balanced. Suppose AB, CD are the original chromosomes AB and CD are then normal, BC and AD give a translocated gamete.

STEWART The concept is introduced simply to explain the report of Grumbach *et al* (1957) in which you have a chromatin positive and a chromatin negative case in a sibship.

FORD Yes I appreciate that it is a very ingenious suggestion and I think it is well worth looking into I just wanted to point out this one difficulty

JOST I wish to comment just on one point of detail in Dr Lennox's presentation He suggested that sex reversal of the gonad in the positive Klinefelter should occur very late in development. If we agree with the idea that gonadal masculinizing substances are required to masculinize the genital tract in these patients, we must assume that the gonadal sex reversal occurred very early before the beginning of the sexual differentiation of the genital tract Various experiments on animals show such early—even if incomplete—sex reversal.

COLOUR VISION IN KLINEFELTER S SYNDROME

P M F BISHOP D.M., F.R.C.P.,
M A. FERGUSON-SMITH, M.B. Ch.B.
B LENNOX, M.D Ph.D., M.R.C.P
P E. POLANI M.D M.R.C.P D C.H and
J S S STEWART M.B Ch.B
(Read by P E. POLANI)
Guy Hospital, London, and Western Infirmary Glasgow

THIS communication is a preliminary report on Colour Blindness in Klinefelter s Syndrome Last year Dr Maurice Lessof Dr Peter Bishop and I tried to find confirmatory evidence on the genetic maleness of some patients with gonadal dysplasia by checking their colour vision (Polani *et al.*, 1956) In a group of 25 such patients, of whom 20 were chromatin negative 4 chromatin positive and 1 unknown we found 4 who showed a major colour vision defect as tested with the Ishihara Tables We thought that this could be interpreted as evidence that these patients had only one X chromosome. We suggested that this method should be applied to other groups of patients with a discrepancy between apparent sex and nuclear sex.

In a subsequent letter to the *Lancet* (Bishop *et al* 1956) we invited workers, who had cases of Klinefelter s syndrome, to stud them by this method in addition to nuclear sexing hoping thereby to pool enough clinical material to permit a statistical analysis of colour vision in male hypogonadism

The response was quite good Further cases are in the process of being tested by various workers but you might like to see the results to date Over half of the cases in Table I were studied at Glasgow by Dr Ferguson-Smith, Dr Lennox, and Dr Stewart. A good proportion of the rest were seen at Guy s, and we are grateful to the colleagues who reported to us ind idual cases Dr Prader of Zurich Dr Wrong of Manchester Dr Clarke of Li erpool, and Drs Wh te Joseph, Norman and Carter of London

Among the chromatin negative cases those labelled "other are

patients with azoospermia or extreme oligospermia, abnormally small testes and testicular biopsies that are not typical of the Klinefelter syndrome but show predominantly either fibrosis or absence of germ cells, or a mixture of both conditions

We can accept the incidence of colour blindness in the general population of this country as detected by the Ishihara Test, to be 7–8 per cent among the men and not more than 0·6 per cent among the women Then the expectation of major colour defect in a sample of 19 females would be 0·11 approximately and in a

TABLE I

COLOUR VISION TESTED BY ISHIHARA IN KLINEFELTER'S SYNDROME"

	Number Tested	Normal	Major Colour Defect
Chromatin positive—Observed	9	9	
Expected	9·00	8·89	0·
Chromatin negative—Typical	6	3	3
Other		9	
Total—Observed	26	22	4
Expected	26·00	24·00	2·00
All cases	45	4	4

sample of 26 males about 2 By the way we might add to these patients, if we so wish under the heading other the two brothers with familial testicular deficiency described by Sohval and co-workers in 1959 and re-described in 1956 whose colour vision was normal and who were chromatin negative.

I will hasten to add that our results are not statistically significant at the conventional level but I think they show an interesting trend I hope you will not object to my showing to you these data, though incomplete We need results on many more cases. And, later we shall need to have more precise information, by means other than the Ishihara Test on the type of colour vision anomaly present in our groups. Professor Pickford in Glasgow kindly tested two of the colour blind patients on the anomaloscope and found them both deuteranomalous. I may add that Professor Wright saw the Ishihara results of three of our ovarian agenesis cases and thought that they too were probably deuteranomalous.

In conclusion should the necessary extension of our data show this same trend one plausible interpretation of the findings could be that the chromatin positive cases have in fact an XX sex chromosome constitution as has been suggested the inference

being that some patients with Klinefelter's syndrome are genetic females

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DISCUSSION

HUGGETT Is there any history of hæmophilia?

POLANI We have not seen any cases but we are keenly looking for one. It has been reported in the literature in one sibship in a case of male hypogonadism

SACHS Did you do finger-print patterns in the Klinefelter series? Did you find anything in favour of the same sort of thing as you suggest for the colour vision?

POLANI No we have done palm-prints and finger-prints of very few cases of Klinefelter's syndrome, and I wouldn t like to say anything about them.

PRADER I would like to ask an additional question about feeble mindedness in Klinefelter s syndrome An Argentine paper I think, has described feeble mindedness occurring very frequently in Klinefelter s syndrome. From our much smaller experience we have the same impression. I would like to know if anyone knows whether feeble mindedness is less frequent in chromatin negative cases and more frequent in chromatin positive cases. It would be of great practical assistance in discovering these cases much earlier than is possible now

POLANI Generally speaking the typical Klinefelters have minor degrees of intellectual impairment. I wonder if Dr Ferguson-Smith has anything to add on this point?

FERGUSON-SMITH We found that many of our chromatin positive patients were of low intelligence. I wondered if possibly this was the "upper crust of Klinefelter's syndrome and in fact I might find more among people who were really mentally defective and who were in mental homes. I went to one of our big mental institutions with 800 male patients and with one of the staff went rapidly round the wards and picked out some random samples of cases which showed some form of hypogonadism. We really wanted quick results and in our first thirty using buccal smear techniques, we picked out one I was very excited about this and went back to do some more and found another this was a couple of weeks ago The interesting thing about this is that attached to this hospital is a school for mentally defective children of the educable type. Possibly this will provide a good chance of finding a pre pubertal case, which has not been described previously

and which will probably throw great light on the pathogenesis of the testicular lesion. (See Ferguson-Smith M A. (1958) *Lancet* I 928) The two cases which were chromatin positive were we think typical cases of this syndrome They both had small testes and one aged 26 had gynæcomastia. The other incidentally was a passive homosexual.

SEX REVERSAL

THE PRESENCE OF OVARIAN TISSUE IN AN INDIVIDUAL OF MALE NUCLEAR SEX

D J B ASHLEY M.B Ch.B
David Lewis Northern Hospital, Liverpool

This case is the first, which can be traced in which the gonads of an individual of male nuclear sex showed the full histological features of ovaries including oogonia and Graafian follicles.

CASE REPORT

An only child was referred to Alder Hey Children's Hospital at the age of 10 days because of doubts concerning the true sex. The external genitalia consisted of labia with a vaginal opening and a phallus suggestive of an enlarged clitoris on which there was a hypospadiac urethral orifice. There was in addition a communication between the urethra and the vagina. Otherwise she was a small but apparently normal baby with no evidence of skeletal abnormalities. The urinary 17 ketosteroid excretion was within normal limits. This finding together with the absence of signs of virilization was regarded as excluding adrenal cortical hyperplasia. Laparotomy at the age of 17 months revealed a normal sized uterus and Fallopian tubes; there were two ovaries in the normal position which were slightly enlarged and cystic. There was no evidence of any testicular tissue retroperitoneally in the inguinal canals or in the labia. Section of part of the right ovary showed a follicular cyst; many oogonia were present; formed Graafian follicles were scanty.

Smears of buccal mucosa and a section of skin showed the nuclear sex to be male; in no instance did more than 3 per cent of the cells contain "sex chromatin".

Blood films were also examined. The incidence of satellite bodies on the polymorphonuclear leucocytes was of the female type.

DISCUSSION

This case presents several unusual features. The phenotype is undoubtedly feminine despite the abnormal development of the

urogenital sinus and the genotype, as shown by the nuclear sex, is male The finding of a female frequency of satellite bodies on the polymorphonuclear leucocytes need not be regarded as a contradiction of the skin sex, as I have demonstrated that the sex chromatin mass on the nuclear membrane is not identical with the satellite body of the leucocytes (Ashley 1957) and similar discrepancies have been observed by Professor Greenblatt in other cases of gonadal dysgenesis

The only cases in which a female phenotype has been associated with a male genotype have been those of gonadal dysgenesis (Turner's syndrome) This condition is commonly associated with skeletal and cardiovascular anomalies which were absent in this case and a few instances have been recorded in which there was anomalous development of the urogenital sinus (Gordan *et al* 1955 Grumbach *et al* 1955) The histological features of the gonads in this syndrome are however different from those in this case the characteristic picture is that of a fibrous structure resembling ovarian stroma without germinal elements The histological appearance of the gonad has led to the currently accepted theory of the aetiology of Turner's syndrome that it is due to failure of the embryonic, undifferentiated gonad to develop either because of some unidentified environmental factor (Grumbach *et al.*, 1955) or because of failure of the germ cells to migrate in a normal manner to the urogenital mesenchyme (Segal and Nelson, 1957) with the assumption of a neuter form which, in man is considered to approximate to that of the female Neither of these explanations is tenable in this case, and a state of sex reversal analogous to that seen in Klinefelter's syndrome with female nuclear sex must be postulated.

The morphogenesis of the condition can most easily be explained by the hypothesis of Hoffenberg and Jackson (1957) that the nature of the gonad and thence the development of the Müllerian and Wolffian duct systems and the structures derived from the urogenital sinus is normally determined by an evocator itself genetically determined and that in this type of case as in Klinefelter's syndrome the evocator is abnormal to the genetic sex.

This case completes a sequence of developmental abnormalities of the gonad female to male sex reversal (Klinefelter's syndrome) — true hermaphroditism with gonads of both sexes and genotype of either—male to female sex reversal

It will be of great interest to follow the progress of this child to see whether in the years ahead she is functionally as well as anatomically female and especially whether she becomes pregnant.

(*I am indebted to Dr R. H White-Jones and Miss I M Forshall for permission to report this case which is to be published in collaboration with Dr C H Jones*)

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DISCUSSION

POLANI I wish I could answer the question implied in Dr Ashley's last remark. I am a pediatrician and this, coupled with the fact that I have this interest in nuclear sex, means that when I see a child who is undersexed I have the nuclear sex determined. Now there were two girls who came to our out patients about the time when the Turner's syndrome was occupying us very much three years ago, and who were very small. Of these two girls one was mentally defective dwarfed, and she looked bizarre. She didn't look like a Turner's syndrome by any means and she had retinitis pigmentosa. The other was bright and cheerful and had no somatic stigmata of any sort in fact apart from the fact that she was short she was perfectly normal in every way We did the skin sexing of both and the blood sex, and both turned out to be chromatin negative. We thought they were cases of ovarian agenesis or whatever you like to call the condition, and we left it at that, waiting for development. One of them was referred, aged 14, to Dr Bishop, the other was not. Now it turned out that at about the age of puberty both girls developed first breasts, then pubic hair and axillary hair and then started menstruating and they continued regularly for the last year in spite of having this chromatin negative sex of both skin and blood. We don't feel justified in having a look inside though it is very tempting and I don't think it would be permitted.

OVERZIER I think as long as you did not slice through the whole gonad in this case it is not proved that it is not a true hermaphrodite.

DAVIDSON I wonder if perhaps Professor Barr has thought about this in relation to the true Klinefelter cases. He sent me blood films from cases in which he found a normal "female

frequency of nodules in the skin but only a very low frequency of drumsticks in the blood. Is it not possible that one might find another type of case in which there was a normal frequency of drumsticks in the blood but a low frequency of nodules in the skin?

BARR There were two cases of Klinefelter's syndrome in the one we both had to examine 800 in the other 900 neutrophils to find six typical drumsticks—but as far as the oral smear and epidermis was concerned these appeared like normal females. The sex chromatin was present in the same frequency and as far as one could judge the same size and shape and everything else I think we cannot answer that problem until we know exactly what is the relationship between the drumsticks and the sex chromatin in other cells.

KLINGER I think we had a similar example at Basle where buccal mucosa and epidermis were female in a case of Klinefelter's syndrome Blood films were sent to Professor Wiedemann and we got back a count of 6 drumsticks to 2000 or 1500 cells.

SIEBENMANN May I answer the question put about the frequency of the sex chromatin in Klinefelter's syndrome? We have found a low incidence of chromatin positive nuclei in the Sertoli cells We know also that others have found intermediate values between 7 and 70 per cent. But we were not able to find more than 6 7 or 8 per cent in those cases where the Sertoli cells were in a good condition and I would like to ask Professor Barr if he hasn't made a similar observation There was one case in which the Sertoli cells in some tubules showed 60–68 per cent chromatin positive nuclei and others were completely devoid of chromatin positive cells It seemed to me also that the percentage in other organs adrenal cortex for example, was lower than those percentages Professor Barr has found in normal females. But that might be a consequence of a different technique or that this material is not very suitable for this study

BARR I think we are agreed that the Sertoli cells are either unsuited for this cytological detail or for some other reason the frequency of the nuclei with the sex chromatin In cases which have otherwise female nuclei is less than normal. I don't recall having done any recorded counts but certainly looking at the tubules one does get that impression. I also get the impression that even the Leydig cells are probably a little patchy with those that show the sex chromatin and those that do not. As far as oral mucosa and skin are concerned they appear to be normal female in the few that I have seen

POLANI On this point I think it was Sohval who pointed out this very fact in the Sertoli cells. Could I ask one of the embryo-

logists whether they would tell us what the homologue is in the female of the Sertoli cells? It would be interesting to know what their nuclear chromatin is.

? I think it is generally believed to be the granulosa cell.

APPLICATION TO THE STUDY OF TUMOURS

CHAIRMAN PROF MURRAY BARR

20

THE NUCLEAR SEX OF TERATOMAS

LOIS M. MYERS M.D. Ph.D., D.M.R.T.
Ontario Cancer Foundation, London Clinic, Victoria Hospital,
London, Canada
formerly Middlesex Hospital, London

In this paper I shall present the findings of a study of the nuclear sex of a group of teratomas. As a basis of comparison a preliminary examination was made of a variety of normal tissues, including skin, fibrous connective tissue, all component elements of ovaries and testes, and 3 to 4-month-old human embryos. In addition, a detailed study was made of 162 benign and malignant non-teratomatous tumours. I shall not discuss these findings except to say that *in some tumours from females, sex chromatin was* frequently free in the nucleoplasm or adjacent to the nucleolus for example, in Brenner tumours of ovary up to 86 per cent of the masses were in these positions. I have therefore designated as sex chromatin any regular discrete chromatin mass approximately 1 μ in diameter with the appropriate chemical constitution, irrespective of its position in the nucleus.

One hundred and five teratomas 64 from females and 41 from males, were examined. All *suitable component* tissues were studied including when present, stratified squamous epithelium and skin, nervous tissue smooth muscle fibrous connective tissue cartilage, respiratory thyroid and intestinal epithelium. Multiple areas each consisting *of 100* consecutive suitable nuclei of the particular cell type were examined, and the incidence and position of regular chromatin masses recorded these masses were Feulgen-positive, stained with methyl green and differentially with crystal violet and thionin

Of the 64 teratomas from females, 47 were ovarian 7 mediastinal, 6 sacrococcygeal and 4 from other sites 47 were benign and 17 malignant. In the benign group, typical female sex chromatin was readily identified uniformly throughout the tumour in all component tissues, in an average of 60–65 per cent of nuclei with the majority of the masses situated at the nuclear membrane (Tables I and II). There was, however some variation in position for example in well-differentiated stratified squamous epithelium in 9 ovarian teratomas, 2/3 of the sex chromatin masses were free in the nucleoplasm or adjacent to the

Table I

TERATOMAS FROM FEMALES—AVERAGE PERCENTAGE INCIDENCE OF SEX CHROMATIN IN VARIOUS TISSUES

Site	Total Number of Cases	Stratified Squamous Epithelium	Nervous Tissue	Smooth Muscle	Fibrous Connective Tissue	Cartilage	Respiratory Epithelium	Intestinal Epithelium
Ovarian	47	67	56	68	64 6	62·0	58·3	57
Mediastinal	7	75	—	68 8	63 9	6 5	68 7	69
Others		73	68 6	72 6	7 9	65 8	64 8	65

Various tissues not present in all cases.

Table II

TERATOMAS FROM FEMALES—AVERAGE PERCENTAGE INCIDENCE AND POSITION OF SEX CHROMATIN

Site	Number of Cases	Average Total Percentage Sex Chromatin	At Nuclear Membrane	Free in Nucleoplasm	Adjacent to Nucleolus	Multiple Masses
Ovarian	47	6	53	4 8		
Mediastinal	7	67·8	57	5 8	4·7	3
Others		68 8	58 7	5	3	1 8

Abbreviations used in subsequent tables
S.C.—Sex chromatin N.M.—At Nuclear membrane F—Free in nucleoplasm N—Adjacent to nucleolus M.M.—Multiple masses

TABLE III

TERATOMAS FROM MALES—AVERAGE PERCENTAGE INCIDENCE OF SEX CHROMATIN IN VARIOUS TISSUES

Group		Total Number of Cases	Stratified Squamous Epithelium	Smooth Muscle	Fibrous Connective Tissue	Cartilage	Respiratory Epithelium	Intestinal Epithelium	Cuboidal Epithelium	Non-specific Stratified Epithelium
Testicular teratomas	Group A	3	3	9	4·4	6·4	4·3	4·6	5·3	5·5
	Group B		7	73·6	63·2	67·3	—	64·7	6·6	66·3
Mediastinal teratomas		5	3·6		4	5·5	4·3	4	6	—
			82	7	73	67	64	69	—	—
Other teratomas			4·5	4	3	7	5	4	—	

Table IV

TESTICULAR TERATOMAS (GROUP B)—AVERAGE PERCENTAGE INCIDENCE AND POSITION OF SEX CHROMATIN IN VARIOUS TISSUES

Tissue	Number of Cases	Average Total Percentage S.C.	N.M.	F	N	M.M.
Stratified squamous epithelium	7	7	40	9	8·3	0·7
Smooth muscle	5	73·6	66·6	5·4	4	
Fibrous connective tissue		65·[illegible]	46	·8	6·4	
Cartilage	3	67·3	53·7	8	5·6	
Intestinal epithelium	9	64·7	47·[illegible]		6	0·4
Cuboidal epithelium	7	6[illegible]·6	43·3	9·4	7·7	[illegible]
Non-specific stratified epithelium	4	66·3	5[illegible]·5	6	7	8
Average	—	67·	49·9	9·	7·[illegible]	0·9

nucleolus In the malignant teratomas, sex chromatin could not be identified accurately in neuroepithelium, embryonic liver and kidney or in malignant glandular epithelium. Immature mesenchymal and proliferating fibroblast like cells contained typical sex chromatin at the nuclear membrane in approximately 2/3 of nuclei.

Of the 41 teratomas from males, 33 were testicular 6 mediastinal, 1 retroperitoneal and 1 from side of neck.

According to their nuclear morphology the 33 testicular teratomas were divided into four groups

A	Nuclear sex uniformly male	13 cases
B	Nuclear sex uniformly female	10 cases
C	Nuclear sex diverse—male and female	8 cases
D	Nuclear sex abnormal female	2 cases

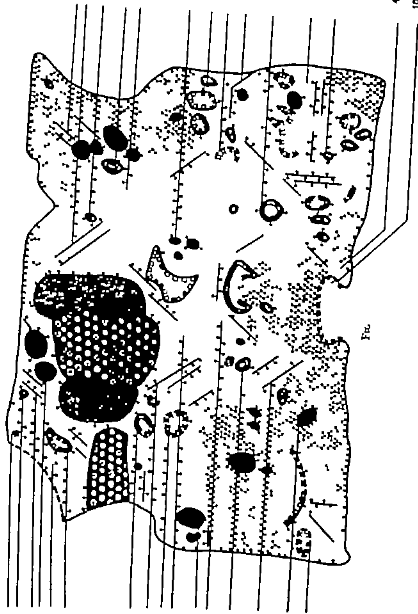
Group A (nuclear sex uniformly male) In 13 cases, nuclei from well-differentiated tissues had a typical male morphology with sex chromatin present in approximately 5 per cent of nuclei (Table III)

Group B (nuclear sex uniformly female) In 10 cases, chromatin masses identical with female sex chromatin were found in approximately 2/3 of nuclei in all suitable well-differentiated tissues the majority of the masses being situated at the nuclear membrane (Tables III and IV) There was slightly more variation in size of the sex chromatin in the testicular teratomas than in the ovarian group

Group C (nuclear sex diverse—male and female) In 8 cases, the nuclear morphology varied in different portions of the teratoma, being typical male with a low incidence of sex chromatin in some areas, and equally typical female with a high percentage of sex chromatin in other regions (Table V) Multiple areas of various tissues of comparable degrees of maturity were examined and percentage counts made

To illustrate this In Case No 1 the male and female components were clearly separated in the two sections available one section containing entirely female type nuclei and the other section male type nuclei except for one female region separated from the main portion by a zone of dense fibrous tissue. In the male component, the average incidence of sex chromatin was 3 3 per cent in 7 tissues, whereas in the female component typical sex chromatin was present in an average of 75 per cent in 6 different tissues.

In Case No 2 the male and female areas merged imperceptibly (Fig 1) The male component contained sex chromatin in an



Key to Fig 1

Stratified squamous epithelium

Non-specific stratified epithelium

Intestinal epithelium

Cuboidal epithelium

Smooth muscle

Cartilage

Malignant glandular epithelium

Embryonic nervous tissue

Fibrous connective tissue

Haemorrhagic areas

FIG. Diagram of section from teratoma (Case No) showing the percentage of chromatin positive nuclei in different tissues.

average of 6·1 per cent of nuclei in 6 tissues while the female component had sex chromatin in 67·8 per cent of cells. In the remaining six tumours in this group the *male* and *female areas*, with a low and high incidence of sex chromatin respectively were clearly integral parts of one tumour Figures 2–9 illustrate representative nuclei from various tissues in the male and female regions

It is possible that the first tumour in this group is composed of two separate teratomas, but it is inconceivable that all the *teratomas with this diverse nuclear morphology are fused multiple* growths.

Group D (nuclear sex abnormal female) In 2 cases, one-third to two-thirds of nuclei contained two chromatin masses each indistinguishable from normal female sex chromatin as illustrated in well-differentiated smooth muscle (Figs. 10 and 11) and mucous-secreting intestinal epithelium (Fig 12) as well as in *benign connective tissue fibroblasts and immature stratified* squamous epithelium (Table VI)

Of the remaining eight non testicular teratomas from males, seven had male nuclear morphology and one mediastinal teratoma contained female sex chromatin uniformly in all tissues (Table III)

There is, as yet, no adequate explanation for the presence of chromatin *masses* identical with *female* sex chromatin in teratomas from males. Various possibilities will be considered briefly

Many investigators including Leuchtenberger *et al* (1954) Stowell (1946) and Bader (1953) have found a wide variation in

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FIG. Testicular teratoma, Group C. Stratified squamous epithelium—male component No sex chromatin visible. H. and E. 800.

FIG 3. *Testicular teratoma, Group C.* Intestinal epithelium—female component. Sex chromatin free in nucleoplasm. H. and E. 800.

FIG. 4 Testicular teratoma, Group C Benign connective tissue fibroblast —male component. Sex chromatin is not visible Feulgen × 1800.

FIG. 5. Testicular teratoma, Group C. Benign connective tissue fibroblast —female component. Typical sex chromatin at nuclear membrane H. and E. × 800.

FIG. 6. Testicular teratoma, Group C. Smooth muscle—male component. No sex chromatin present. *H* and E. × 800.

FIG. 7 Testicular teratoma, Group C. Smooth muscle—female component. Typical sex chromatin at nuclear membrane H. and E. 800.

FIG. 8. Testicular teratoma, Group C. Cartilage—female component. Sex chromatin visible in nuclei. H. and E. 800.

FIG. 9. Testicular teratoma, Group C. Ciliated respiratory epithelium—female component. Sex chromatin nuclear membrane Feulgen 800.



Fig.

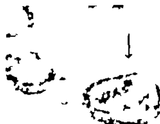


Fig. 3

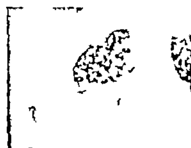


Fig. 4

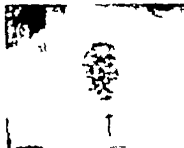


Fig. 5



Fig. 6



Fig. 7

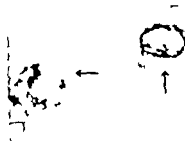


Fig. 8

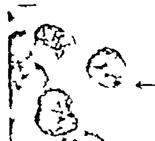


Fig. 9

Table V

TESTICULAR TERATOMAS (GROUP C)—AVERAGE PERCENTAGE INCIDENCE AND POSITION OF SEX CHROMATIN IN VARIOUS TISSUES IN MALE AND FEMALE COMPONENTS

Case Number	♂ Component						♀ Component					
	Types of Tissue	Average Total Percentage S.C.	N.M.	F	N	M.M.	Types of Tissue	Average Total Percentage S.C.	N.M.	F	N	M.M.
	a, b d, g, h	3 3	9		0·4	—	a, b f h	75	49 7	3 3	1·2	8
	a, b, d, f h	6·	5	7	4	5	a, b d f g	67 8	3 7	9 6	4·8	7
3	a, b f g h	3 3	2		7	3	a, b f h	50·5	35 4	4·6	8	2·5
4	b f g	5	·9	4	4	3	b f g	58·4	56 5	6	0·3	
5	d	9	4 5	5	5	5	b d	63·7	49·3	5	3	6·4
6	d	7	4·9	9	3	—	d	47·4	37·2	6·5		5
7	b d, f h	3·7		·4		0·3		78	5	2	4	7
8	d, f	4 8	3		5	—	a, f	57·7	44	6 3	6·7	0·7

Abbreviations: a—stratified squamous epithelium
—fibrous connective tissue
—respiratory epithelium
g—cuboidal epithelium
b—smooth muscle
d—cartilage
f—intestinal epithelium
h—non-specific stratified epithelium

the DNA content of malignant tumour cells which has been attributed to disturbances of nucleic acid synthesis or to alterations in the chromosome content of cells associated with mitosis

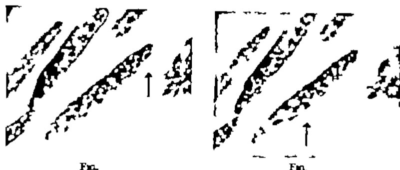


Fig. Fig.

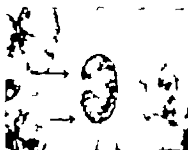


Fig.

Fig. 0. Testicular teratoma, Group D Smooth muscle—sex chromatin visible at nuclear membrane. H. and E. 800.

Fig. Field identical with Fig. 0, at different level of focus. A second sex chromatin mass is visible at nuclear membrane H. and E. 800.

Fig. a. Testicular teratoma, Group D. Intestinal epithelium—a sex chromatin masses visible at nuclear membrane. H. and E. × 800.

Koller (1943 1947) Hsu (1954a 1954b 1954c) and Hsu and Pomerat (1953) have described abnormal mitoses in human tumours with resultant alteration in numbers of chromosomes in cells Evidence has been presented by Swift (1950 1953) and supported by Thomson *et al* (1953) that DNA is synthesized during interphase, so that a cell contains double the normal amount of DNA prior to any morphological changes associated with mitosis it is theoretically possible that this might occur in the disorderly growth of teratomas, with resultant fusion of the

Table VI

TESTICULAR TERATOMAS (GROUP D)—AVERAGE PERCENTAGE INCIDENCE AND POSITION OF SEX-CHROMATIN LIKE MASSES IN VARIOUS TISSUES

Case Number	Types of Tissue	Average Total Percentage Sex chromatin-like Masses	Single Chromatin Mass				Two Chromatin Masses				Three Chromatin Masses
			Total Percentage S.C.	N.M.	F	N	Total Percentage S.C.	N.M. (F or N)	* N.M.	± F or N.	
1	*a, b, c, d, f*	77·8	45	3	7·4	6·6	3[illegible]·6	5·4	9·6	7·6	0·2
	b g	[illegible]8·3	40·8	35·5	*	3·3	37	4·8	*	*	5

heterochromatic portions of the XY chromosomes to produce a mass similar to female sex chromatin. It is unlikely that enlargement of the XY chromosome complex itself, as has been described by Barr and his co-workers in nerve cells of the cat under abnormal conditions (Barr *et al.*, 1950 Barr and Bertram, 1951 Crouch and Barr 1954 Lindsay and Barr 1955) would produce such prominent chromatin masses in teratomas.

On rare occasions, a teratoma might develop in an apparently normal male whose tissues contain female sex chromatin for example in Klinefelter's syndrome and related conditions, as described by Plunkett and Barr (1956a 1956b) and Grumbach *et al* (1957) It is interesting that Sohval and Gaines (1955) found female sex chromatin in a malignant mediastinal teratoma from a 22 year-old male with marked bilateral testicular atrophy Further Hunter and Lennox (1954) found female sex chromatin in a pineal teratoma from an 8-year-old boy but subsequent examination of a skin biopsy raised considerable doubt about the nuclear sex of the patient. In my own group of extratesticular teratomas from males, one of eight contained female sex chromatin this was a mediastinal teratoma from a 14 year-old boy and in the sections available, there was some doubt whether a portion of thymic tissue which contained sex chromatin was actually part of the tumour or not. The evidence was inconclusive and no further tissue or skin biopsy has been available for examination.

This would not explain the relatively frequent occurrence of female sex chromatin in the testicular teratomas, for in my series of 93 cases, none contained significant chromatin masses in any cell type in the compressed rim of testis, with the single exception of the Wolffian duct derivatives. In both normal and atrophic testes, the efferent ducts contained one or two Feulgen-positive chromatin masses similar to female sex chromatin in an average of 56·5 per cent of nuclei one-quarter of these were adjacent to the nuclear membrane. There is, however no evidence that teratomas originate from these cells.

In an attempt to explain the occurrence of female-type nuclei in teratomas from males, Hunter and Lennox (1954) and Lennox (1956) have advanced a theory involving self-fertilization of two haploid cells (gametes or others) and Tavares (1955a 1955b) has suggested the parthenogenetic division of haploid cells followed by chromosome reduplication. In view of the diverse nuclear morphology found in approximately one-third of testicular teratomas I think that some further explanation must be sought.

(*Reports giving the full details of these nuclear sex studies in normal tissues and teratomata are to be published (J Path. Bact in press)*)

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DISCUSSION

SACHS I wonder why the speaker feels she must look for any further explanation than one which has already been suggested I would have thought that the evidence which she has presented would tend to support the suggestion of parathenogenesis, because since one cannot tell the difference by the nuclear sexing method between XY and XO the mosaic picture can very well be explained by the existence of an XX part and an XO part. You can get such a thing in a male because if you have a parthenogenetic development of a cell which has an X chromosome either you will then get an XX teratomata or (if some of the cells have lost one of the X chromosomes) you will get an XX and an XO in the same tissue. In the same way in the cases in which there were two large sex chromocentres could you not explain this simply by the fact that it is known that there are tumours which

are polyploid? Since these polyploid tumours have duplicate chromosome material, they would also have a duplicate number of sex chromosomes and therefore you could quite readily get two sex chromatin dots in these tetraploid teratomata. On this last point, of course it would be relatively simple to see whether the nuclear size in the teratomata with two distinct chromocentres is greater than the normal nuclear size with one chromocentre

MYERS I have not examined nuclear size in detail I think the impression is that they are larger Regarding the initial statement about XX and XO I think that could well fit in but what I could not understand was the merging of the two are imperceptibly

KLINGER If Dr Sachs is willing to accept polyploidy a explaining two sex chromatin bodies in an originally male nucleus why not say that it is just as possible that a single sex chromatin body in male nucleus is also due to polyploidy—not requiring the parthenogenetic theory at all. In other words, if you have a single X chromosome and through polyploidy you get two of them then you could have a single sex chromatin body and if this goes on you could have two and possibly more bodies.

LENNOX In some male teratomata with well-formed single sex chromatin masses the nuclear size is the same as in normal squamous epithelium.

SACHS The question of the two bodies as Dr Myers said is that where she had two bodies the nuclei were larger You need not expect them to be double the size You could show that there was more chromosome material present by determining that there was more nucleic acid present. It is a relatively simple assumption to make that if you have more chromatin material, that some of the chromatin material present in excess is the sex chromosome material. I have never looked at teratomata myself but judging from your photographs the size of the two sex chromatin masses was normal and there was no question of its being divided into two halves.

MYERS I have not done any detailed measurement of them but they do appear perhaps slightly larger—particularly in fibroblasts which contain two chromatin masses.

SACHS It is known from work with all kinds of other tumours that you can get enormous variation in chromosome number in different tumours You can have diploid, tetraploid, and polyploid tumours.

MYERS My feeling is, that is more likely to be the explanation of this variation in teratomata rather than going back to the origin of them from parthenogenesis or something of that sort.

BARR If polyploidy occurs in a male there will still probably be a Y chromosome which may inhibit the X

KLINGER I don't know whether I quite agree that the Y inhibits the X, or that there is no half-sized sex chromatin in the male nucleus I think you yourself Professor Barr have stated that you may see a small mass in the male nucleus I have been able to see it too The problem is that it is so small we cannot with certainty differentiate it from the rest of the particulate chromatin. There may be a half-sized chromatin mass in the male nucleus and I don't see why if the male nucleus with its one X is polyploid, in which case you would have two Xs or more that it cannot then develop a sex chromatin body

BARR There is one point against that I think, that comes out of the study of tumours other than teratomata, and that is that in tumours arising in male hosts, in our experience, one does not find the sex chromatin and some of those must surely have more than one X chromosome

ATKIN Anticipating what I will say about tumours other than teratomata, I would like to say now that we have measured the DNA content of our male tumours although from the DNA content they were tetraploid, none of them had sex chromatin masses.

BARR In our work on tumours, our main difficulty has been to get really good preparations It is difficult to get specimens put into a suitable fixative quickly enough. One other question Were the two tumours which had two sex chromatin masses highly malignant?

MYERS No they were not. In the two sections available there was scarcely anything which would suggest malignancy at all and they looked histologically benign.

BARR The reason I raise that is that in malignant tumours in female hosts, in certain specimens up to 15 per cent of the nuclei had two typical masses of normal size

LENNOX In defence of our original suggestion of the fusion of two haploid cells it does seem to me to have one big advantage in that it affords a possible explanation of the energy of growth of the teratomata. The fusion of two haploids does seem to be a stimulus to subsequent growth whereas there is no reason at all why the mere parthenogenetic division of a cell should cause active growth. There are arguments the other way but I think there is still something in our original idea

SIEBENMANN I want to ask Dr Myers about the constituents of the ovary She said she had examined the different parts, and yesterday the question was raised of the sex chromatin in the Sertoli cells and in the perhaps analogous granulosa cells.

MYERS I found that the sex chromatin was readily identifiable in granulosa cells and in all the elements of the ovary The only confusing part was in the Wolffian remnants again and they have the peculiar picture that the afferent ducts of the testis have, with one or two chromatin masses in the nucleus, quite often free or adjacent to the nucleolus Apart from this, all the tissues in the ovary contained typical sex chromatin.

DAVIDSON It seems to be rather important that where we find female teratomata in males, that other tissues, completely different should be examined because in one case we think we may have picked up a junior case of Klinefelter's syndrome. The blood was chromatin positive as well as the tumour At least a blood film should be examined in these cases.

MYERS In most cases one only has the tumour to examine In some of the extratesticular cases it is possible that the tumours might be arising in cases of Klinefelter's syndrome

21

FURTHER ENQUIRIES INTO THE SEX OF TUMOURS

AMÂNDIO S TAVARES M D
Department of Pathology Faculty of Medicine
University of Porto, Portugal

THE study of the sex chromatin in tumours has yielded some interesting results, the interpretation of which takes for granted an intimate correlation between this mass of chromatin and the presence of material from the XX chromosome pair

The method, as proposed by Barr and his school may now be considered as generally accepted, once a few technical conditions are obeyed and its extensive use in intersex cases and, especially in tumours—whose alterations due to the multiple processes of differentiation and dedifferentiation are well known—induces us to review the notion of its value outside the normal cell

It is not my intention to deal here with intersex the problems which arise from the study of intersexuals will appear with higher intensity in the tumour field As regards the nuclear sex of tumours it must be said that the difficulty of comparison between *lesions* classified by different investigators using different criteria adds to the difficulty inherent in the observation of cells possessing a more or less aberrant cytophysiology

Hunter and Lennox (1954) found that tumour tissues do not differ as to this character from normal tissues some teratomata from men are to be excluded from this general rule This report has been confirmed by others (Cruickshank, 1955 Moore 1955 Moore and Barr 1955 Tavares, 1955a, b Rodermund 1956 Rivière 1956 etc.) and the teratomata exceptions were used in the elaboration of teratogenetical hypotheses, always under the assumption that a chromatin positive nucleus means a female, XX cell.

Later irregularities were found within this pattern Weinmann *et al* (1955) reported some female basal-cell carcinomata in men and conversely loss of sex chromatin was observed in a fair number of cells in undifferentiated-cell carcinomata from our files (Tavares, 1956 1957) Another exception to the rule of host tumour sex identity was pointed out in prostatic carcinomata by Coutts *et al* (1955 1956) who used phase contrast for their

observations and concluded that chromatin-negative tumours were more responsive to hormonal therapy than chromatin positive ones and to conclude the list of irregularities discovered, we will mention the report of some chromatin-positive seminomata in men (Mancini 1956 Rivière 1956)

It was thought that these apparent exceptions ought to be discussed and compared with my personal experience since they seem to re-open the problem of the visualization of the sex chromatin within the nucleus, and eventually the problem of the true relation of this chromocentre and the XX chromosome pair (relations which others are better qualified than I to define)

In the case of undifferentiated-cell carcinomata, sometimes, as already reported (Tavares 1956 1957) the nuclear pattern is greatly altered, an embryonic pattern being substituted for the adult one and a tendency towards intermediate values may appear the frequency of chromatin positive nuclei being smaller than usual in female tissues or greater than usual in male tissues. It must be said that the concept of undifferentiated-cell carcinoma —as distinct from the anaplastic carcinoma—is vague based as it is on the absence of nuclear or cytoplasmic differentiation and of any tendency to glandular or malpighian evolution. The nuclear structure and the gradation of frequencies found in these instances enable us, I think, to speak of a sex chromatin behaviour rather than of an hypothetical, difficult to explain sex reversal

Although these undifferentiated-cell tumours are included in the general group of basal-cell carcinomata, Weinmann's findings cannot be compared with those reported as accompanying cellular undifferentiation the counting technique is more restrictive than that we use, with lower sex chromatin frequencies in normal male tissues Weinmann *et al* (1955) considered as "female any neoplastic area with 2 or more per cent of chromatin positive nuclei, and thus they reported 58 per cent of positiveness among the 180 areas counted in the 11 neoplasms studied in men The real percentages varied between 2 and 30 per cent (mean 13) for males, and the average for tumours from women was 34 per cent, i e. about half the normal value however it seems hardly justifiable to correct the obtained frequencies by multiplying them by a factor of 2 giving to Weinmann *et al* values (theoretical values) of 50 per cent or more for tumours from men It is essential to identify the reason for the low frequencies in neoplasms from women before such a correction may be applied

After the presentation of this paper the author was informed that Moore and Barr while studying the sex chromatin of malignant cells from ... omas, were led to suspect that "the incidence of sex chromatin was more likely to be low in the undifferentiated tumours, but the nature of our material did not lend itself to ... clear demonstration of this (Barr 1957)

Furthermore, the irregularity of distribution of chromatin-positive nuclei is well known to anyone who has determined the nuclear sex of a tumour. As Rodermund (1956) stresses, this phenomenon may be due to alterations of the DNA metabolism, and further work is needed to throw light on this point.

The altered cellular physiology is certainly responsible for the greater difficulty and lack of precision in identifying the nuclear sex in malignant tumours than in normal tissues or in benign tumours. Such is Rivière's (1956) explanation for the high frequencies found in cancers from men and the loss of sex chromatin from malignant cells from women. However, the values reported by this investigator form two homogeneous groups, separated from each other by a short hiatus centred at 30 per cent and besides "it is true that, if only appropriate nuclei had been examined and included in these statistics, it would be possible to attain the average of 50 or more per cent, in all cases of malignant lesions from women".

Low values are frequently encountered in tumours with high mitotic indices,* but it is not yet possible to find any rule for this fact. There are many exceptions and technical conditions (for example, variability of the section thickness and of the cellular volume) which inhibit the use of the appropriate statistical treatment. Even if evidence of correlation is found, it is possible that this correlation may be attributed to a common cause rather than to any interdependence. The frequency of mitoses influences directly the frequency of resting nuclei and consequently the possibility of expression of the chromocentre. It is true, on the other hand, that the method of selecting nuclei for examination somewhat reduces the effect of this influence, since dividing nuclei are not included in the count.

As regards the cases of high frequencies in male tumours, Weinmann *et al.* think that the appearance of sex chromatin is probably due to the same cause in all cases and may be "a manifestation of an internal or external causal factor which may be simultaneous with, but may also precede assumption of frankly neoplastic characteristics". Thus, all cells presenting an altered nuclear sex would eventually give rise to tumours. The reason for the low frequencies of chromatin positive nuclei remains still to be discovered (since such an alteration would be indispensable for the appearance of the tumour, there should be a greater percentage of chromocentres), as well as why such a fact is not observed in other non teratoid tumours.

* The cases of great reduction of sex chromatin frequency—4·66, 4·30, and 4·0 standard deviations from the mean, as calculated for differentiated-cell tumours (Tavares, 1955a, b)—are those with a high mitotic index.

As already stressed the concept of undifferentiated-cell carcinoma is somewhat cloudy and calls for a sharpening of defining criteria, and also it would be necessary to compare the criteria used by those working in this field However the fact remains that, among the tumours, which were at first considered as exhibiting the same nuclear sex as the patient, some exceptions exist which may point to a common morphological type of tumour or at least, to an identical alteration of cytophysiological processes.

Since we know that cellular differentiation is associated with a variation of chromosomal complement in multiples of *n*, it might be that the cytological characteristics of undifferentiated-cell carcinomata represent the morphological evidence of such a variation, in the direction of haploidy for females, or polyploidy in the male cases. It would be interesting to identify the time pattern of the alteration of the chromatin positiveness. If this is transient and non-inheritable, then these tumours should be considered as variants of the basal-cell carcinoma a permanence and inheritance of the nuclear sex alteration would, on the other hand be an argument in favour of this being a nosological entity

Disturbances of the differentiative processes, other than de-differentiation seem to have no influence on the sex chromatin frequency Two types of tumours were investigated mixed tumours, most of them of the salivary glands, and ovarian neoplasms. The choice of this material was based on the presence of multiple differentiation lines in mixed tumours and the de-differentiation observed in many ovarian tumours. Seventy mixed tumours were chosen at random, selected for good fixation condition and observed by the usual technique (Tavares, 1955a, b) The average values obtained 72·6 (range 56–82) in 39 tumours from women and 9·0 (range 2–23) in 21 tumours from men, suggest that only a few of the tumours from men tend to copy the nuclear pattern described for some undifferentiated cell carcinomata with a high frequency of sex chromatin. This may be related to disturbances of cellular nutrition, since it is more apparent in areas of mucoid differentiation On the whole however it seems that the study of the nuclear sex enables us to corroborate the view of those authors who think that mixed tumours are not teratoid in nature

One hundred and ten tumours of the ovary (12 dysgerminomata and 98 carcinomata) were studied next. In 2 cases of carcinomata low frequencies of chromatin-positive nuclei were found (52 and 51 per cent) mitoses being abundant in only one of them. All the other tumours were easily classified as "female" (average 69·9 range 56–83)

Another possible source of influence on the chromatin positiveness of nuclei is the particular moment of cellular physiology in which the nucleus is observed Sexual chromatin counts on cortical adrenal cells were compared with the levels of steroid (17 ketosteroids, 17 hydroxycorticosteroids, and total reducing steroids) urinary excretion prior to operation, and no evidence of correlation was obtained. Values of r were found to oscillate between 0·04 and 0·08 for 22 degrees of freedom. The material (adrenals from 8 men and 16 women) came from patients with Cushing's syndrome and women with breast carcinoma who were unilaterally adrenalectomized

The relationship between the chromocentre and sex has led some investigators to study the nuclear sex of tumours which are markedly influenced by oestrogens Sohval and Gaines (1955) reported the absence of sex chromatin in 5 adenomata and 3 adeno-carcinomata of the breast (2 and 1 respectively from men) *but these results ought to be revised since their material seemed* to be unsuitable for sex chromatin studies Hienz and Ehlers (1957) examined 30 carcinomata of the breast from women as well as 3 from men, and attempted to correlate the nuclear sex with the responsiveness of these tumours to oestrogens and their metastasizing capacity

Examining between 200 and 500 nuclei in each case, and using the frequency ranges of 14–30 and 0–2 5 as defining the sexes, Hienz and Ehlers (1957) admitted that sex inversion had occurred in about one third of these tumours, and in both sexes.

In another type of cancer sensitive to hormonal influence, the prostatic carcinomata, Coutts and Inzunza (1955) reported the existence of both chromatin positive and chromatin-negative cells, and used the difference to foretell the response to endocrine therapy Later Coutts *et al* (1956) advanced the hypothesis of the Mullerian origin of the chromatin positive prostatic lesions

However the method used by the Chilean investigators does *not permit, in my opinion an exact examination* phase contrast observation, although a truer method than those using fixation of tissues may give a rather imprecise idea of the chromatin pattern and a poor definition of the sex chromatin The mean values reported by Coutts *et al* for both sexes are very near to each other the maximal frequency reported is 39 per cent, and 8 per cent is considered as a 'feminoid' value.

Phase contrast may be used, I think, in the examination of cells from the oral and vaginal mucosæ, but care is needed in discarding unsuitable cells, and observation by a standard method is always to be recommended Preliminary results suggest that this technique

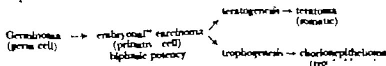
may be of value as a routine procedure, with the condition that only well-spread nuclei be used. Cells from prostatic carcinomata rarely fulfil this condition.

On the other hand, 50 carcinomata of the prostate were selected from the many in our files (nuclear structure was seldom adequately preserved). In none of them could the sex chromatin be detected, confirming the report of Hienz and Ehlers (1957) concerning the chromatin negativity of these tumours.

Of an altogether different significance and worthy of some attention are the results obtained in testicular tumours. Rivière (1956), for instance, found some chromatin-positive seminomata (2 out of 6) and suggested that these may be considered as the end point of a seminomatous evolution of a teratoma. For Mancini (1956), pure seminomata are always negative, but pseudo-seminomata may be positive. Since the pseudo-seminoma is formed by elements similar to seminal cells and areas of embryonic carcinoma (Mancini, 1956), the positiveness is attributed to seminoid differentiation in a teratoma. This confirms the nosological independence of this type of tumour which Schnyder (1952) had already defined by the high gonadotrophin production, absolute resistance to irradiation, high malignancy, and an age incidence similar to that of teratomata. We have as yet no means of identifying a pure seminoma evolved from a teratoma, other than by sex reversal, and one might ask how many of the chromatin-negative seminomata have such an origin.

I was able to study 12 pure seminomata and found every one of them to be chromatin-negative and, in one instance, with coexistence of seminoma and teratoma, both tumours were chromatin negative. It must be said that this discrepancy between my findings and Rivière's report is not a real one. Statistics fail when the criteria and the classification vary and are defective, and we must remember the doubts expressed by Friedman (1951) and by Willis (1948) on this subject.

Based upon the previous observation by Moore of transitions between germinomatous and embryonal-carcinomatous elements in tumours of the testis, and his own observation on the possible dysembryomatous evolution of metastases from a pure (or so considered) germinoma, Friedman states his belief in the relationship between the seminoma and the teratoid neoplasms thus:



It is interesting to point out that 3 out of 5 chorionepitheliomata and 2 of 4 embryonal-carcinomata of the testis which were included in this investigation presented typical sex chromatin bodies, i e all the chromatin-positive tumours of the testis from our files are found to the right of Friedman's scheme This calls *for a fundamental alteration during the transition from dysgermi* noma to embryonal carcinoma, if one accepts such a relation between these neoplasms

As regards teratomata, Hunter and Lennox (1954) reported a possible sex difference between the tumour and the patient in men and suggested a conjugation of gametes, or at least of haploid cells as a mechanism of teratogenesis. Later using the results reported by them Cruickshank (1955) and myself statistical evi dence was obtained of a mechanism involving one single haploid cell (Tavares, 1955a) This evidence is stronger if we include Moore and Barr's and Rivière's 58 teratomata of the testis, 32 of which were chromatin-positive, and 9 teratoid tumours form our files not previously reported (Table I Hypothesis III)

TABLE I
TERATOMA IN MEN

Nuclear Sex	Obs	Hypothesis I (ratio) Exp.	χ^2	Hypothesis II (ratio) Exp.	χ^2	Hypothesis III (ratio) Exp.	χ^2
+	49	[illegible] 5	3[illegible] 60	29 67	[illegible] 593	44 5	455
	40	66 75	7 80	59 33	6 [illegible]	44 5	455
Total	89	89	4 [illegible]	89	8 89	89	9
		P	000,000,00[illegible]	P =	000,0 4	P	34

Obs. Observed frequency Exp. Expected frequency

Unfortunately teratomata in men are scarce and although they are more frequent in women these tumours are not of any use for the present argument since they are all chromatin positive Another point which should always be kept in mind when working with both teratoid and non teratoid tumours is that the basis for the haploid-cell hypothesis still assumes that chromatin-positive nuclei possess *at least two X chromosomes.*

A completely different line of approach to this problem is the immunological study of teratomata of the ovary by the isolation or the demonstration of ABO blood group substances. This is difficult and has many possible drawbacks, among which two are

really important the possible contamination of the teratoma by group substances from the host's blood and the possibility of cells coming from adjoining ovarian follicles. The method is worth trying and makes use of material from women which hitherto could not be used in the investigation of this problem. If the haploid cells which conjugate are the ovum and its first polar body in a heterozygous host we will be dealing with two genetically different cells and conjugation will lead to a teratoma with the same antigens as the host. If parthenogenesis occurs a teratoma with only one of the host's antigens will be produced.

I would have liked to have presented data from this line of approach, but no fresh teratomata have become available and techniques were prepared for such a study and fixed material cannot be used in serology since blood group substances are destroyed by formalin. The method is open to anyone who wishes to try it and I would advise that a teratoma from a group AB woman be employed for clear-cut results as the detection of A and B substances is much simpler than that of O (or H).

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DISCUSSION

This paper was discussed with paper 22. See page 173.

22

OBSERVATION ON SEX CHROMATIN IN HUMAN TUMOURS

N B ATKIN M.B., B Ch., D M R T
Department of Cancer Research, Mount Vernon Hospital, Northwood, Middlesex

We are engaged at Mount Vernon Hospital in a study of the chromosome complement of human tumours, our objects being to *find out what variations occur and to see if these variations can* in any way be correlated with the pathological and clinical features of the tumours, for instance their response to radiotherapy

Our data have consisted mainly in chromosome counts and measurements of the deoxyribonucleic acid (DNA) content of individual nuclei.* If from a study of the nuclear chromatin pattern we can deduce the presence or absence of the XX chromosome pair we have a further potentially useful means by which we can characterize the tumour cells.

Unfortunately in many undifferentiated tumours the presence of multiple chromocentres of varying sizes renders the identification of the typical "sex chromatin" structure difficult or impossible *in many of the cells. In the present investigation therefore* detailed study has been confined to tumours in which the presence or absence of the sex chromatin could be clearly ascertained in at least half the nuclei

The observations have been made on squash preparations such as are familiar to cytologists, stained in aceto-orcein and in some cases by the Feulgen method also These preparations are excellent for showing the detailed chromatin pattern and we also have the advantage that only whole nuclei are observed

The typical "sex chromatin" is seen as a peripherally-situated usually somewhat elongated chromatin structure a little over one micron in its long axis, and with a central pale area In some tumours it appears more prominent than in others but it is difficult to be sure whether this represents a real difference in terms of mass or of amount of DNA. It can be followed into

* DNA measurements ha been made photometrically in collaboration with Mr R. M. Richards t the Wheatstone Physics Laboratory King College Strand.

early prophase, but later in prophase when the chromosomes first become distinguishable as individual threads all the chromosomes appear isopycnotic. (Figs. 1, 2 and 3.)

Table I gives the findings in five different tumours in females. Although this investigation is concerned with malignant tumours,

TABLE I
SEX CHROMATIN IN A SAMPLE OF CELLS FROM FIVE INDIVIDUAL TUMOURS IN FEMALES

Case Number	Site	Histology	Number of Sex Chromatin Bodies ( )			Multiple Chromo-centres	Number of Nuclei Counted
1	Bladder	Papilloma	3	97	5		200
2	Corpus uteri	Well-differentiated tall columnar-cell mucus-secreting adenocarcinoma	·0	92·3	3·9	7	481
5	Rectum	Moderately well-differentiated columnar-cell adenocarcinoma	7·5	4	74	4·5	200
4	Corpus uteri	Tall columnar-cell well-differentiated adenocarcinoma with areas of squamous metaplasia	94	6			325
3	Cervix uteri	Poorly-differentiated squamous-cell carcinoma	82	5		5	82

52 per cent with no prominent chromocentre 30 per cent with prominent nucleolus-associated heterochromatin.

a papilloma of the bladder has been included for purposes of comparison Sex chromatin was present in 97 per cent of the nuclei (Case 1)

The second case, a well-differentiated carcinoma of the uterine body was unusual in having a modal chromosome number of only 28 with a correspondingly low modal DNA value (It should be mentioned that DNA estimations on a wide variety of human tumours suggests that the great majority have a near diploid hyperdiploid, or near tetraploid chromosome number) Although this tumour had lost a fair proportion of its chromosome complement, it would appear to have retained the XX pair (or at least their heterochromatic portions) since a single sex chromatin body was seen in 92 per cent of interphase nuclei. A few of the nuclei contained two sex chromatin bodies this was a fairly constant finding in tumours in which a single body was seen



Fig.



Fig.



Fig. 3



Fig. 4

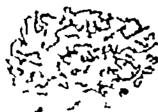


Fig. 5



Fig. 6

Fig. Well-differentiated papillary adenocarcinoma of the ovary. Single sex chromatin in majority of nuclei. (× 550.)

Fig. Same case as Fig. Region with nuclei having sex chromatin bodies. (× 550.)

Fig. 3. Moderately well-differentiated adenocarcinoma of endocervix. Early prophase (× 750.)

Fig. 4. Moderately well-differentiated carcinoma of the rectum (Case 3). Two heterochromatic regions in interphase (lef) and early prophase (right) (× 800.)

Fig. 5. Case 3 mid-prophase (× 500.)

Fig. 6. Case 9 metaphase (× 300.)

in the majority of nuclei and it seems likely that these are tumour cells which differ from the basic (most commonly-occurring) cell-type in having duplicated some or all of their chromosomes, including the XX pair They sometimes appear to be located at random, but also occur at times in aggregates of 10–20 or more cells, suggesting that a cell with double sex chromatin may be capable of at least a limited amount of cell division

In a few tumours two peripherally situated sex chromatin



Fig. 7 Fig. 8

Figs. 7 and 8. Poorly-differentiated squamous cell carcinoma of the cervix Juxta-nucleolar heterochromatin structure (550)

bodies were seen in the majority of nuclei This is illustrated by case No 3 a moderately well-differentiated carcinoma of the rectum. This tumou had a modal chromosome number of 60, and a correspondingly raised modal DNA value it would appear therefore to have undergone duplication of part of its chromosome complement, including the XX-pair Figs. 4 and 5 *show two prophases from this tumour In each of which two con*densed chromosomal regions, presumably derived from the two sex chromatin bodies, can be seen. The criticism might be made that the piece of tumour examined is unrepresentative of the tumour as a whole It should be noted, however that in this case *it was possible to obtain specimens on four separate occasions over* a period of several months, two obtained by biopsy and two at operation. In each case a similar pattern of sex chromatin was seen Of the other tumours characterized by two sex chromatin bodies, three had DNA values consistent with a tetraploid *chromosome number*

The last two cases are representative of a further small group in which *no* sex chromatin was seen in the majority of cells. It

is not clear however whether this indicates absence of the XX pair A typical sex chromatin was certainly seen in a few cells in these cases. Sometimes (e.g case No 5) an apparently double chromatin structure lying in contact with a nucleolus was seen (Figs 7 and 8) In unsquashed nuclei however a tubular structure was quite frequently visible By focusing up and down this could be followed from a nucleolus to the periphery In some instances at least it appeared to communicate with the exterior and might therefore be formed by the nuclear membrane dipping inwards to a nucleolus. It was associated with heterochromatin which of course might or might not be derived from the X chromosomes (Figs. 9–14)

In Table II are summarized, according to site, all the cases seen. Typical sex chromatin was present in some cells of all the female cases, but for those included in the first column the picture in the majority of cells was obscured, as already mentioned, by the frequent presence of irregular chromatin condensations.

TABLE II
SEX CHROMATIN IN MAJORITY OF CELLS OF [illegible] HUMAN MALIGNANT TUMOURS (FEMALE)

	Not Determined				Total
Cervix uteri	43	[illegible]		5	[illegible]
Corpus uteri	6				[illegible]
Breast	5		—	—	5
Vulva		3	—	—	4
Vagina	—	—		—	
Ovary			—	—	
Bladder	—		—	—	
Caecum	—	—	—		
Colon	[illegible]		—	—	4
Rectum	—			—	3
Other sites	5	5	—	—	8
	59	50	7	8	[illegible]

TUMOURS IN THE MALE

Carcinomata arising at various sites	—	—	—	[illegible]	[illegible]

In all the male tumours examined the absence of sex chromatin could be clearly determined in the great majority of cells These cases include some tumours which from DNA estimations were tetraploid.

To summarize although tumours may occur in the femal which have lost one or both X chromosomes it is not considered that we have any good evidence either for or against this in our



Fig. 9

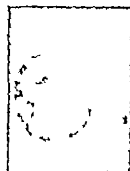


Fig.

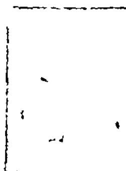


Fig.

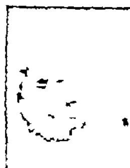


Fig.

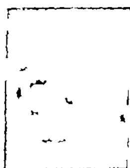


Fig. 3

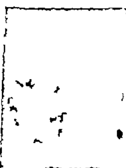


Fig. 4

Figs. 9– 4 Same case as Figs 7 and 8. Successive optical sections through an unsquashed nucleus. An apparently tubular structure can be traced from nucleolus, seen in the upper part of the nucleus in Fig. 9, to the periphery (400)

(All the photomicrographs are of aceto-orcein preparations, using Baker oo [illegible] objective)

material. However the presence of two sex chromatin bodies in the majority of cells of some female tumours strongly suggests the duplication of the XX pair in the stem-line of these tumours.

DISCUSSION on papers 21 and 22

BARR I think anyone who has worked with malignant tissues can well appreciate the difficulties involved (which Dr Atkin certainly must have had) in being certain that a particular

contained that kind of element it was so malignant that I could not sex it, but I think Dr Myers has had more experience than I have.

MYERS I would not have sexed those portions.

LENNOX There is one important point that Dr Tavares raised. I think he said there is a special recognizable group of undifferentiated tumours including some of the basal-cell tumours of the skin in which there is an intermediate level of chromatin count. Now it is not just that there are some tumours which you can t count properly—there is a defined group with this peculiar constitution If that is true it is a rather remarkable observation and I don t think it ought to go without notice I would like to know what Dr Atkin thinks about it. I am sure his method is much more satisfactory than the use of sections but on the other hand, the material Dr Atkin used perhaps did not include any of these special tumour types.

ATKIN I have only looked at five or six basal-cell carcinomata and they have always been in keeping with the sex of the subject. I would like to add that variations in the sex chromatin have not been correlated in any way with the size of the tumour or histology apart from the fact that undifferentiated tumours tended to have multiple chromocentres in females.

BARR Do you think the undifferentiated tumours have a lower incidence of nuclei with recognizable sex chromatin?

ATKIN I don t think my observations would support that

GENERAL DISCUSSION ON SYMPOSIUM

SEAR A small contribution, which is a practical use of the technique of nuclear sexing In the placenta there are certain areas which are ill-defined as regards their source and origin, and one in particular is the placental septum—a thin membrane which divides the cotyledons from one another There has long been an argument as to what its origin was, maternal or foetal. Of course the obvious proceeding is to take male foetuses, and examine the tissue then if it is male it is foetal, and if female maternal in origin. Most of the histologists working both on electron microscopic and histochemical studies have come more or less to the conclusion that it is foetal tissue composed of trophoblastic cells. Now we found quite clearly that it was maternal and although we sometimes got low counts we felt that it was a technical fault more than anything else. In material from Caesarian section where the placenta was very fresh we found high counts of 85 per cent or so I was interested to hear that Mr Klinger and his team have done the same work and have found the same results. It seems that, despite histological evidence this technique has proved finally the origin of this tissue.

KLINGER Not only is the septum of the placenta maternal, but also the islands. What used to be called the chorionic islands arise from maternal cells.

BARR No one during the discussion has brought up the alternative hypothesis advanced recently by Segal and Nelson concerning the possible relationship of sex chromatin and sex chromosomes. (I was just wondering whether Dr Ford or Dr Sachs or someone working with chromosomes would have anything to say about it.) They suggested that the sex chromatin is an autosomal heterochromatic derivative rather than related to the sex chromosomes, containing M genes that are inactive in the female because they are heterochromatic and active in the male because they are euchromatic.

SACHS I remember discussing this with Professor Witschi and I think we parted and agreed to differ I personally would have thought that the most conclusive evidence that the sex chromatin really corresponds to the sex chromosomes was that shown beautifully by Dr Polani and company when they showed, without the

slightest shadow of doubt, that material sexed as male genetically has male sex-linked characteristics and that in point of fact the best supposition to make under such circumstances is that one really is dealing with sex chromosome material. I really don't see what better evidence one can have.

BARR That is certainly very important, and so are your own observations on a larger and smaller chromocentre in the young spinous cells.

FORD I have nothing to add to what Dr Sachs has said but I do think there is an opportunity in the near future through technical advances, of being able to get a much better correlation of the true chromosome picture and the presence or absence of the sex chromatin.

SACHS I am very keen to show whether there is such a thing as an XXY or an XO which will help to straighten the whole thing out There is another thing which one should be able to show in a few days. In cases of the true Klinefelter's syndrome there are a few cases with spermatocytes and spermatogenesis, and here you really can see the sex chromosomes One can look at some of these compare them to a normal male and see then whether they look like an X with a little Y or an XX If one wants any better evidence than Dr Polani gave this will certainly be able to clear up this point very neatly

BARR Using a tissue culture of fibroblasts, as Shaw and Barnard did you could look for a Y chromosome.

PLATT Does Dr Polani's demonstration of sex linked characters in chromatin negative people distinguish between XY and XO? I don't see that it does at all

SACHS But it does distinguish between something that has one X and something that has two Xs.

LENNOX Is there any evidence that an XO constitution exists in man?

HAMERTON No evidence at the moment.

POLANI There is no evidence of any Y linked gene in man and therefore it will be difficult to prove by non-cytological genetic analysis.

SACHS Some Turner's may be XO—I still think there may be something in that.

STEWART Concerning the problem of chromatin positive Klinefelters, I think it is clear that the cases have been selected to some extent. The cases with gross gynaecomastia come up to an endocrine clinic and the cases with infertility come up to an infertility clinic. As far as we know sterility appears to be associated with this condition but spermatogenesis has been described

in our own series, and though I have not seen the actual seminal specimens there were some sperms present. These cases may in fact very rarely be fertile. If they were, they would produce only daughters. I have an experiment to suggest which could only be done on a national scale and that is that we look among large families for one with 10 to 15 all girls, and look at the father and do a buccal smear

SWYER Another possible line which might be followed is to examine the sex of the offspring of men who have begot children but who are known to have very low sperm counts, and see if there is an excess of females among them.

POLANI Two points. The first following on what Dr Stewart has said I contacted the Registrar-General's Office to see if we could track down these really large families of one sex. The difficulty would be that the parents would no longer be available and the children too scattered about the world. The second point is whether it is possible at this meeting to see if we could obtain more information on the colour blindness in Klinefelter's syndrome. I would much appreciate it if anyone with cases could test them with Ishihara charts and tell us the sex chromatin status.

PLATT In reply to Dr Stewart's question These families with all girls would be so very rare that I suppose they would not affect the statistics. The actual statistics are according to Armstead who examined a large number of families of single sex, that there is an excess of families which have all males. The families which have all females, even five, six, seven, and eight and more daughters could all be explained by chance—they fitted the distribution curve of a chance distribution But there is an excess of families with all males. It does not help Dr Stewart in the least, but it is an extremely interesting observation I am sure Dr Armstead would be interested to know if anyone has an explanation of the families which are all males.

STEWART A possible explanation for that is that there is an excess of males at birth—one theory is that the sperm containing Y moves faster than the one containing X

SERR We have been working on the primary sex ratio for some time It is a slow plod because statistically the number requires to be four or five thousand, and if the primary sex ratio comes nearer one to one than the sex ratio at birth of 105 to 100 the number of cases you need goes up. So far at the Carnegie Institute a large series of abortions have been examined on histological tissue from the gonads. That rules out almost all the spontaneous abortions which are in the first or second month before

the gonads are sufficiently differentiated Abortion material can easily be obtained and sexed from the mesenchymal tissue. If a sufficiently large series is available then a statistically significant number as regards the primary sex ratio, will be obtained and thus will give some light also on the difference between the sex ratio at birth and the primary sex ratio.

CONCLUDING REMARKS

MURRAY L. BARR

Before commenting on the excellent papers that were presented yesterday and to-day some thought should perhaps be given to the advisability of convening a similar Symposium at a future time if warranted by further progress in this field. With this purpose in view I suggest that the Committee which organized the present Symposium be asked to continue as a Standing Committee. (There was full agreement on this point.) It has been suggested to me that a small group might be assigned the task of considering a revision of the nomenclature for sex anomalies and also to study the matter of their classification. (Professor Huggett thought that this should be left in the hands of the Standing Committee; his suggestion met with unanimous agreement.)

The following discussion will be of an informal nature; it will consist principally of comments on a few of the points that were raised during the course of the Symposium.*

The variations in the position of the sex chromatin within the nucleus may be worth calling to your attention again. In neurones the favoured position of this chromocentre is against the nucleolus in those species of the orders Carnivora and Artiodactyla that have been studied (Barr *et al.*, 1950; Moore and Barr, 1953), while it is more likely to lie against the nuclear membrane in neurones of monkey and man (Prince *et al.*, 1955; Mylle and Graham, 1954), the only primates whose neurones have been examined from this point of view. Further, the position of the sex chromatin changes to some extent as cells mature (Graham, 1954*a*). The sex chromatin is usually at the nuclear membrane in cells of non-nervous tissues. It may be significant that the pattern in neurones seems to be consistent within a mammalian order. Virtually nothing is known of the forces that act on nuclear structures or influence their position. Variations in the functional states of cells may contribute, in a minor degree, to the difference in the sex chromatin's position from one neurone to another in the same specimen, since its position is clearly altered

* This discussion was illustrated by lantern slides. Most of the illustrations have been published, or will be published, elsewhere so they are omitted from this account. I wish to thank Dr Robertson Smith for the opportunity of reviewing and revising these extemporaneous remarks and adding certain references.—M.L.B.

during chromatolysis (Barr and Bertram, 1951 Crouch and Barr 1954 Lindsay and Barr 1955) However this factor cannot be invoked to explain all of the variations that are encountered and this phenomenon arouses the desire to learn more of intranuclear forces by whatever experimental methods may be applicable to this difficult type of problem.

The accessory body of Cajal is a component of the nucleus of nerve cells that needs thorough investigation. It will be discussed briefly although it is apparently not related in any way to the sex chromatin. My purpose in directing attention to the accessory body at all is derived from our lack of knowledge of its relation to other nuclear structures or its role in the cell's economy The accessory body is a spherical structure that has a mean diameter varying between 0·5 μ and 1·0 μ depending on the type of neurone, and it has identical characteristics in males and females (Thomson *et al* 1957) The accessory body usually lies free in the nucleoplasm it is adjacent to the nucleolus in a few neurones but is adherent to the nuclear membrane with great rarity Unlike the sex chromatin the accessory body is refractory to staining with basic dyes or by the Feulgen method. Also in contrast to the sex chromatin, the accessory body becomes rather smaller in chromatolytic neurones and remains relatively stationary in the nucleus (Haggar 1957) There is rarely any mention of the accessory body of Cajal in papers that deal with nuclear cytology That it has been largely overlooked by cytologists stems, no doubt, from the necessity of using silver nitrate staining methods for its demonstration and these methods are seldom used in the study of chromosomes or other components of the nucleus. The accessory body is likely to have some significance in cell metabolism our ignorance on this point induces me to bring a nuclear component that has no apparent relation to sex to your attention at this time.

More accurate information is required on the percentage of nuclei that contain sex chromatin. Our group may have been remiss in publishing figures for the incidence of nuclei with sex chromatin in different tissues and regions of the nervous system in various species, because there is an understandable tendency to read into tabulated data a greater accuracy than they have The figures simply record what one sees and they depart to varying degrees, depending on circumstances, from the real condition in a population of cells. Difficulties in the way of obtaining accurate data are both technical and observational Whole nuclei are seldom included in sections that are 5 μ in thickness and in spite of all efforts some material from which the figures are derived is

not of that highest technical quality which is so desirable for cytological work. Whether a particular mass of chromatin should be recorded as sex chromatin requires a difficult decision in a few cells. In various tissues of man, we found that the percentage of nuclei with sex chromatin varied between 58 and 88 per cent in the female, and between 0 and 21 per cent in the male (Moore and Barr 1957) These figures were intended only to convey a general impression of what one is likely to find in sections. In terms of whole nuclei, the figures for nuclei in tissues of females are undoubtedly too low In the male masses of chromatin that are recorded as sex chromatin, in order to be as objective as possible are almost invariably smaller than the sex chromatin of female cells and many of them are probably unrelated to nuclear sexual dimorphism More accurate data, in terms of whole nuclei, can be obtained by studying nuclei in whole mounts of thin membranes. For example, sex chromatin was found to occur in 96 to 98 per cent of nuclei of the amnioallantoic membrane of female cat embryos (Graham, 1954b) Another approach is to use fairly thick sections that have been prepared so that nucleoli and chromatin are stained differentially Along this line, Cook *et al* (1951) showed that the sex chromatin could be identified in 95 per cent of cells of the lateral geniculate body of the female cat, after staining with thionin. The study of whole nuclei does not always produce such high figures, as those who use the oral smear method well know This matter has been discussed at some length because we should be cautious in drawing inferences concerning possible variations in chromosome numbers of a tissue cells from the rough data that are now available for the incidence of sex chromatin in a population of nuclei. However when more accurate data are available, the studies on sex chromatin will have a bearing on the question of a possible variation in the diploid chromosome number of certain tissues.

Dr Marberger mentioned to me that she had encountered difficulty in identifying the sex chromatin in nerve cells of cattle I would like to comment on this briefly because we had some experience with bovine material in connection with the study of nuclear structure in the freemartin (Moore *et al* 1957) Certainly nuclei of non-nervous tissues of cattle are not suitable for this type of work because of the coarse nature of the chromatin, and this applies to the smaller neurones as well. However the large neurones such as primary motor and sensory neurones and the larger pyramidal cells of the cerebral cortex, have vesicular nuclei whose sex chromatin is readily demonstrable in females. The nuclei of freemartins are exactly like those of normal females,

which bears out the contention of Tandler and Keller and of Lillie that the bovine freemartin is a partially masculinized female

A small detail but probably an important one deserves special mention The sex chromatin can often be resolved into two components of equal size in preparations of high technical quality We have seen this in smear preparations and in sections of various tissues in different species after staining with hæmatoxylin and eosin, basic dyes and by the Feulgen method. This detail is seen to better advantage in preparations that have been stained by the ribonuclease-gallocyanin method as advised by Lennox (1956) or by thionin following mild acid hydrolysis as suggested by Klinger and Ludwig (1957) Excellent photomicrographs showing the double structure of the sex chromatin will be found in a paper by Klinger (1957) and it is also illustrated diagrammatically in a paper by Sachs and Danon (1956) The importance of this cytological detail lies in the possibility that it may supply a clue to the chromosomal origin of the sex chromatin It is unlikely that the bipartite sex chromatin represents masses of heterochromatin of a single chromosome because every chromosome in the female has a homologous partner and the two members of a chromosomal pair have, it is to be expected, the same heterochromatic properties. This leads to the conclusion, which must be tentative until more information is available that the sex chromatin represents heterochromatic segments of two homologous chromosomes. The demonstration of X and Y chromocentres, by Sachs and Danon (1956) in young spinous cells of human epidermis lends credence to the view that heterochromatic regions of the X chromosomes are responsible for the sex chromatin in cells of females. However the observation that the sex chromatin consists of two parts fits equally well with the intriguing hypothesis that has been advanced by Segal and Nelson (1957) and by Witschi (1957) These authors, it will be recalled suggested that the sex chromatin may be derived from regions of a pair of autosomes that contain male determiners, these regions being genetically inert when they are heterochromatic (females) and genetically active when they are euchromatic (males) On general grounds, a relation between the sex chromatin and the sex chromosomes seems more plausible but the alternative suggestion clearly demonstrates the necessity of retaining an open mind on this problem until it is resolved to everyone's satisfaction. Regardless of which chromosomes are involved we have no way of knowing so far as I am aware, whether the heterochromatin occupies a rather large segment of

the chromosome or whether several smaller heterochromatic regions clump together to form each component of the bipartite sex chromatin. Neither can we guess the most likely place along the length of the chromosome for these particular heterochromatic segments. Are there any data on chromosomal morphology that bear on these fundamental points? (Dr Ford replied that he had not seen any clear differentiation of the somatic chromosomes of mammals apart from some strikingly understained regions close to the centromeres of some chromosomes. He added that differentiated regions were easy to demonstrate in many chromosomes of plants.) The derivation of the sex chromatin is, in my view one of the more crucial problems within the field covered by this Symposium. Until this problem is solved, it would be wise to err on the cautious side when drawing inferences, either practical or theoretical, from the results of tests of chromosomal sex in sex anomalies. It is encouraging to find that experienced cytologists and cytogeneticists are now bringing their attention to bear on these problems.

The foregoing discussion raises the question of somatic pairing of chromosomes According to current thinking on the derivation of the sex chromatin, the homologous members of at least one pair of chromosomes are closely related spatially in the resting nucleus. Somatic pairing of chromosomes has been demonstrated in plants, insects, the newt, and the frog, but such a chromosomal relationship has not been demonstrated in mammalian nuclei, so far as I am aware. This matter is of considerable importance and worthy of serious investigation. It came up for discussion at a recent Canadian Cancer Conference (Barr and Moore, 1957) because somatic pairing of homologous chromosomes and their crossing-over could lead theoretically to a cell lineage whose genotype differs from that of their ancestral cells. (Dr Ford mentioned that this idea had suggested itself to several people. He stated that two groups have designed experiments in which somatic pairing might be demonstrated, using ascites tumours, and that their results should be appearing quite soon)

Reverting to an earlier comment on staining methods, I would like to say that we now use the method of Klinger and Ludwig (1957) routinely for oral smears and find that it is definitely superior to the procedure we followed formerly Their method has two advantages mild acid hydrolysis prior to staining with thionin improves the definition of nuclear detail and, probably of more importance, eliminates the annoying staining of the bacterial flora in smear preparations. Certain practical points may be mentioned in connection with the interpretation of oral smears.

Preparations from chromosomal females are encountered occasionally in which the sex chromatin is smaller than usual or very much flattened against the nuclear membrane. This thin disk like shape has also been seen in the nuclei of sympathetic ganglion cells of girls and women In addition the proportion of nuclei with demonstrable sex chromatin in chromosomal females may be appreciably lower than that found in sections of various tissues. These points, in addition to the necessity of preparations of high technical quality have to be kept in mind when using the oral smear method If the result is at all equivocal one or both of the other methods, i e examination of a blood film or a skin biopsy specimen, should be used. I am still of the opinion that a carefully prepared skin biopsy specimen gives a particularly convincing picture of chromosomal sex. However the other methods will be used more frequently and quite rightly so because the cells can be obtained more easily The neutrophil method of Davidson and Smith (1954) has very interesting research possibilities, in addition to its value in clinical diagnosis, because we need to know the exact nature of the accessory nuclear lobule of female type neutrophils why the incidence of these cells varies as it does in normal females and why there is a discrepancy between nuclear structure in oral epithelium and epidermis on the one hand, and neutrophils on the other in certain endocrinopathies (Briggs and Kupperman, 1956) and sex reversals (Ashley and Jones 1958)

There is insufficient time to do more than touch very briefly on a few points that were raised in the important clinical papers that we heard yesterday or in this morning s equally important papers on tumour cells. The syndrome of gonadal agenesis (or dysgenesis) has no doubt a special significance for the participants in this Symposium, since the confirmation of the prediction of Professor Jost (1950) that a proportion of these patients would prove to be chromosomal males has had a strong influence on subsequent studies of the sex anomalies. On the basis of our interpretation of specimens submitted by clinicians in various centres, and such clinical data as we have for the patients, we find that about 80 per cent of subjects with the syndrome of gonadal agenesis have male-type nuclei This proportion seems to be in reasonably good agreement with the findings of others. The preponderance of patients in this syndrome who are apparently chromosomal males is a puzzling observation possibly the reason for the disproportion will not be known until the ætiology of gonadal agenesis is understood.

With respect to Klinefelter s syndrome the suitability of the

nuclei of Leydig cells for "nuclear sexing" has a practical interest, since there must be large collections of sections from testis biopsy specimens in the major medical centres. The cases could be studied in retrospect, providing that the fixation of the specimens was suitable for detecting the presence or absence of sex chromatin. In our experience, the nuclei of Leydig cells are not quite as satisfactory as those of many other tissues for this work, partly because of their thicker nuclear membranes, but there is no doubt that the information desired can be obtained from Leydig cells in material that has been suitably prepared. The Sertoli cells are much more difficult to work with, although sex chromatin can be identified occasionally in their nuclei in patients with the Klinefelter syndrome and female type nuclei elsewhere. The finding of nuclei with a female pattern within seminiferous tubules is of interest in connection with the recognition of mature spermatozoa in a few sections of tubules of these patients (Ferguson-Smith and Munro 1958). Now that we know that the pathogenesis of Klinefelter's syndrome (those with female-type nuclei at any rate) probably begins at an early stage of embryonic development, it follows that the pathological characteristics of the gonads are likely to be present long before puberty. A 15 year-old boy is the youngest patient with Klinefelter's syndrome and female type nuclei for whom I have had the opportunity of studying a testis biopsy specimen. In this instance, tubular sclerosis was extremely advanced (Barr 1957). Tests of chromosomal sex on boys with small testes will direct attention to patients in the younger age group (Bunge and Bradbury 1957) and information on the pathology of the gonads throughout life should be available eventually. The work of Professor Jost and other experimental embryologists provides a solid basis for consideration of the pathogenesis of sex anomalies, but more work bearing on their etiology is urgently needed. Dr Sachs and his collaborators (Danon and Sachs, 1957) have influenced us in the direction of considering genetic factors very seriously, and the report of Stewart and his collaborators at this Symposium, on family studies and Rhesus antigens in connection with Klinefelter's syndrome illustrates some useful approaches to the application of genetic principles to sex anomalies.

Apart from the difficult questions that have arisen from the study of nuclei in teratomata, the observations on the nuclei of malignant tissues generally are consistent with the chromosomal abnormalities that have been demonstrated in malignant cells. In our experience the frequency of nuclei with sex chromatin is low in comparison with normal nuclei in about one-third of

malignant tumours in female hosts (Moore and Barr 1957) The nuclei of malignant cells present certain difficulties, partly technical and partly because of their abnormal structure and in my view it is advisable to proceed cautiously with their interpretation, as Dr Myers Dr Tavares, and Dr Atkin have done in this morning's session

Professor Jost formally thanked our hosts during the dinner last evening In concluding these comments, I wish to reinforce his remarks on behalf of all participants, with very special thanks to Dr Davidson and other members of the staff of King's College Hospital Medical School

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